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Workshop on Small Cell Anaplastic Carcinoma of the Lung

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The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1983.

ANNOUNCEMENT

The Editorial Board of *Cancer Treatment Reports* has received inquiries regarding what action was taken to validate the accuracy of the data in the recently published article, "Treatment of Advanced Prostate Cancer With Cyclophosphamide, Doxorubicin, and Methotrexate." These inquiries were prompted by: (1) the recent debarment of Dr. Marc Straus, the primary author of the article, from eligibility for Department of Health and Human Services financial assistance and from eligibility for the receipt from the FDA of Investigational New Drugs for a period of 4 years; and (2) the appearance that some of the experiments reported in the article might be connected with the clinical studies in which deficiencies sufficient to justify debarment were found. At the time of the submission of the article, the debarment action had not yet been taken, but the Board was aware of the serious allegations against Dr. Straus and of the ongoing investigation into these allegations. Because some of the experiments reported in the article appeared to be connected with the research under investigation, the Board took the unusual step of asking Dr. Straus to obtain independent validation of the raw patient data contained in the article through an investigator appointed by the Dean of the New York Medical College.

In June of 1982, the editorial office received a letter from a faculty member at the New York Medical College stating that he had reviewed the data sheet and confirmed the author's findings. Subsequently, the article was accepted for publication and was published in the October issue of *Cancer Treatment Reports* (Vol. 66, No. 10). It has now come to the Board's attention that it failed to note that the data audit was conducted by a faculty member chosen by the author, without knowledge of the Dean, and that original patient charts had not been reviewed, as had been requested.

Therefore, the Editorial Board's initial doubts about the validity of some of the patient data have not been resolved. The Board has notified the authors, and hereby notifies the scientific community, that these doubts will remain unresolved until there is an acceptable independent validation of the raw patient data. Notice of any such validation will, of course, be published in *Cancer Treatment Reports*.

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Workshop on Small Cell Anaplastic Carcinoma of the Lung

Coordinators: Heine Hansen
Joseph Aisner

Ashford Castle, Ireland
September 7–12, 1981

Introduction

Considerable progress has been made within the last decade in the overall knowledge of the biology and management of small cell anaplastic carcinoma resulting in four to five times prolongation of median survival, improved quality of life, and establishment of cures in 5%–10% of all patients. In spite of these advancements, it has become evident in the last couple of years that the therapeutic advancement has reached a plateau and major problems exist in analyzing present available data.

As a consequence, a workshop was held at Ashford Castle, Ireland, September 7–12, 1981, with small cell anaplastic carcinoma of the lung as the subject. The meeting was arranged by the International Association for the Study of Lung Cancer (IASLC) and 45 investigators from 15 countries participated.

The purpose of the meeting was to review the state of the art and to focus on areas of general agreement, and especially on areas of disagreement, in order to identify and stimulate areas of fruitful research. The importance of a close connection and coordination of basic and clinical research was stressed at the meeting. In addition, the necessity of performing large-scale controlled clinical trials was emphasized. The latter became obvious from the reviews, in which it was revealed that many published reports on therapy lack sufficient information concerning pretreatment prognostic factors, histopathologic classification, staging procedure, staging, statistical analysis, toxicity, etc, precluding firm comparison and conclusions from the studies.

Meeting participants were separated into five working groups focusing on details in: (*a*) biology, (*b*) prognostic features and staging, (*c*) the role of chemotherapy, (*d*) the role of radiotherapy, and (*e*) the complications of treatment. The meeting consisted of plenary sessions on these general areas with critical reviews and discussions of main topics and related subjects.

The results of these sessions were then summarized for each area of discussion in order to form a consensus of opinion regarding past, current, and potential research. The five reports which follow represent the consensus of this meeting only and do not represent the official consensus statement of the National Cancer Institute. Furthermore, not every participant necessarily agreed to all points raised in the various groups. Rather the consensus was reached and is presented to further stimulate response from other investigators as well as new concepts for clinical trials. To this extent and on behalf of all the participants we would welcome comments from readers, perhaps in the form of letters to the editor. It will be evident from the five reviews offered here that much further work is necessary to continue to improve upon the initial gains made in the understanding and treatment of this disease.

The workshop was made possible by financial support from Bristol International, Eli Lilly International, Carlo Erba-Farmitalia, Scandinavia and Boel Foundation, Denmark.

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Staging and Prognostic Factors in Small Cell Carcinoma of the Lung

Kell Østerlind,[1,*] Daniel C. Ihde,[2] David S. Ettinger,[3] Richard J. Gralla,[4] Karl Karrer,[5] Stephen Krauss,[6] L. Herbert Maurer,[7] Mikael Rørth,[1] Sverre Sörenson,[8] and Ronald Vincent[9]

Staging procedures are necessary before initiation of therapy of any tumor with metastatic potential. The extent of disease must be evaluated if surgery or irradiation is intended. Recognition of any metastatic lesion is less important if the main therapeutic modality is to be combination chemotherapy. This may presently be the case in small cell carcinoma of the lung, since there is no compelling evidence from prospective randomized clinical trials that any stage-dependent treatment, such as chest irradiation plus combination chemotherapy in patients with localized tumor, leads to improved survival. Nonetheless, many institutions currently prescribe treatment on the basis of extent of disease, and staging procedures are required to assess the degree of tumor dissemination. Staging is also important for estimation of prognosis and for comparison of results from different therapeutic trials. For these reasons a standardized staging system is needed.

Two systems are available for staging of lung cancer. One is the TNM system as described by the American Joint Committee on Cancer Staging and End-Results Reporting (1) and by the International Union Against Cancer (UICC) (2). The second system, introduced by the Veterans Administration (VA) Lung Cancer Study Group (3), includes only a "limited" stage and an "extensive" stage. The TNM system is especially feasible in surgical series (4). The majority of patients with small cell carcinoma of the lung will be classified as having stage III disease and, thus, they will be beyond resectability (5). In 35%–45% of the patients, however, all clinically apparent tumor is confined to one hemithorax, the mediastinum, and the supraclavicular nodes and can be encompassed in a tolerable radiotherapy portal. Such tumors conform to the definition of limited disease in the VA staging system. This explains why the latter system is generally preferred in the treatment of small cell carcinoma. Specific diagnostic methods are not included in the staging systems. Thus, allocation of patients to various stages may be altered by new methods.

These problems have prompted reviews, discussions, and updating of the staging systems (6–8). The following paragraphs contain the main conclusions from a group discussion in the International Association for the Study of Lung Cancer workshop dealing with current staging procedures.

The group developed three sets of staging recommendations: (*1*) those for the rare potential candidate for surgical resection; (*2*) those for patients included in therapeutic trials; and (*3*) those for patients receiving individual therapy. A common staging system has only minor relevance to the third situation. Trials including surgical resection are few. Routine examinations generally will be insufficient to prove operability. The TNM system (1,2) is recommended in this situation. It is detailed and is therefore well-suited to describe the many staging data obtained both preoperatively and at the thoracotomy. The second situation has by far the greatest impact on current research in small cell carcinoma, so the subsequent review will be restricted to staging procedures for patients included in nonsurgical trials.

STAGING SYSTEMS

The classification of patients into two categories, those with limited disease and those with extensive disease, is still recommended. Thus, patients with tumors restricted to one hemithorax, including hilar and medias-

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tinal nodes but excluding pleura, are characterized as having limited disease. Whether patients with contralateral hilar or supraclavicular lymph node metastases or those with multiple ipsilateral pulmonary tumors should be characterized as having limited disease has not been standardized. Precise definitions should be mentioned whenever therapeutic results are presented. A recommended set of staging procedures is summarized in table 1. These procedures are discussed in the following sections.

LIMITED DISEASE SITES

Intrathoracic Tumor

An ordinary chest radiograph is required for evaluation of therapeutic effect and is an essential part of the diagnosis. Tomography may be useful, but this procedure is generally not necessary at the initial clinical staging.

Computerized tomographic (CT) scanning of the thorax has been applied in staging of small cell lung cancer (9). This method enables an estimate of tumor volume and thus a more precise evaluation of response to therapy. Moreover, the extent of thoracic disease as evaluated by use of CT scan has prognostic value (10). This factor may be insignificant when combined with other factors (11), but data to confirm this are lacking. With regard to thoracic metastases, CT scanning may lead to too many false-positive findings (12). The method has improved planning of radiation therapy but therapeutic results are not yet available. So far the method is not generally recommended in staging of the disease.

Bronchoscopy and often mediastinoscopy are fre-

TABLE 1.—Staging procedures: recommendations and comments

Site	Procedure	Recommended	In special situations	Experimental	Comments
Primary tumor	Chest X-ray	+			
	Tomography		+		
	CT scan		+	+	Lacking specificity (ref 10)
	Fiberoptic bronchoscopy	+			
Mediastinum	Chest X-ray	+			
	Tomography		+		
	Mediastinoscopy		+		
	Gallium scintigraphy		+		
Pleura	Chest X-ray	+			
	Cytology	+			Of effusions
	Thoracoscopy		+		
Supraclavicular glands	Fine-needle aspiration or biopsy	+	+		At least cytologic confirmation is recommended
Bone marrow	Marrow aspiration and biopsy	+			
	Bilateral biopsies		+		At least cytologic confirmation is recommended
	Scintigrams		+		
Liver	Peritoneoscopy and biopsy		+		
	Ultrasonography and biopsy		+	+	
	CT scans		+	+	At least cytologic confirmation is recommended
	Scintigrams		+		
CNS	CT scans		+		
	Scintigrams		+		
	Lumbar puncture		+		Positive cytology
	Myelograms		+		
Skin and lymph nodes	Fine-needle aspiration	+			At least cytologic confirmation is recommended
Retroperitoneal organs	Scintigrams			+	Too time-consuming
	CT scans		+	+	
	Ultrasonography		+	+	At least cytologic confirmation is recommended

quently required to obtain a histologic diagnosis. Cytologic diagnosis may be specific (13,14), but at least two positive specimens are recommended. With the use of fiberoptic bronchoscopy, the possibility of reaching the primary tumor has been improved. The method is sensitive in the evaluation of response (15) and is recommended for restaging.

Evidence of tumor invasion into the pleura can be found cytologically or by thoracoscopy. Aspiration of any pleural effusion is recommended, although pleural effusion as the only sign of dissemination may have limited prognostic significance (16). Thoracoscopy is generally not required as a staging procedure.

Regional Lymph Nodes

Mediastinal lymph nodes are involved in most patients with small cell lung cancer (3). However, because of the definition of limited and extensive disease, procedures such as mediastinoscopy or gallium scanning (17) are not required in staging.

The supraclavicular lymph nodes are included in the regional lymphatics of the lungs. Metastases to these glands have no major negative prognostic importance (18,19). Therefore, patients with contralateral supraclavicular lymph node metastases should be classified as having limited stage disease, but this point should be defined in the report.

Histologic or cytologic confirmation of any suspected lymph node (or cutaneous) metastasis is generally recommended.

EXTENSIVE DISEASE SITES

Bone and Bone Marrow Metastases

Radiographs, scintigrams, bone marrow biopsies, and aspirations are options in determining bone involvement. Bone surveys are not sensitive enough to be used in screening procedures (20). Iliac crest bone marrow biopsy and aspiration are recommended to determine bone marrow involvement (21). The number of patients diagnosed as having bone marrow involvement can be increased by 30% if aspirations are performed bilaterally (22). A larger fraction of patients have osseous abnormalities scintigraphically (23,24), but such lesions may not always represent metastases (8).

Bone marrow metastases verified by biopsy or aspiration are significant negative prognostic features (25). Bone involvement is observed in more patients if either scan, bone marrow biopsy, or aspiration is positive, but patients thus characterized do not have a significantly poorer prognosis (11). Some positive scintigrams may, therefore, represent cases without, or with only minimal, amounts of metastatic bone disease. Bone scans should therefore be interpreted circumspectly if they are employed as staging procedures.

Hepatic Metastases

Liver metastases may be diagnosed at peritoneoscopy via biopsy or visualized by use of CT scanning, ultrasonography, or scintigraphy. Liver palpation is recommended. No function test is specific for liver metastases. Serum glutamic oxaloacetic transaminase has a reasonable specificity but is only abnormal in about 60% of patients with liver metastases (26). The test is recommended, however.

Any of the three imaging methods that have been mentioned requires cytologic or histologic confirmation to be specific (27–29), and all have been used as a guide for fine-needle aspirations or biopsies. Ultrasonically directed needle biopsies performed in a consecutive series of 60 patients with solid tumors and ultrasonographic liver lesions resulted in a diagnostic specificity of 90% (30). Data on CT scanning as a guide for needle biopsies are lacking. CT scanning as a screening procedure has been investigated in 66 patients with small cell carcinomas who subsequently underwent peritoneoscopy (31). The specificity was 92% and the sensitivity, 64%. These figures may indicate that smaller lesions can be identified by peritoneoscopy and that embedded lesions in a normal-appearing liver represent a minor quantitative problem. A comparative study including these methods is encouraged.

Because of its inaccuracy and technical problems, scintigraphy is less useful as a guide for needle biopsies. If knowledge of liver involvement is critical in an investigational setting or a specific clinical situation, either peritoneoscopy plus liver biopsy or CT scan or ultrasonically guided needle biopsy is recommended.

Central Nervous System Metastases

Current clinical methods for diagnosis of brain metastases include physical examination and radionuclide or CT scanning. Using these procedures, brain metastases were found in approximately 10% of newly diagnosed patients (32). Most of these patients were symptomatic, and options for specific diagnostic methods were directed by the symptoms. Lumbar puncture and myelography are indicated in the rare cases where meningeal metastases are suspected. The diagnostic value of CT scanning or scintigraphy in this situation has not yet been evaluated. CT scanning is superior to scintigraphy (33) and may result in early diagnosis of asymptomatic cases (34). New high-resolution gamma cameras may improve the radionuclide technique (35). Due to the low incidence of CNS metastases at the time of diagnosis in asymptomatic cases, these methods do not have a major impact on the overall result of staging

(33) when applied to patients without neurologic dysfunction. Since early diagnosis of brain metastases has important therapeutic implications—namely, the need for immediate radiotherapy—further investigation of newer diagnostic techniques is encouraged.

Scintigrams or CT scanning may be useful at restaging, as up to 50% of the patients develop CNS metastases during chemotherapy (32).

Other Sites

The size of the primary tumor as well as the number of metastatic sites imply a negative correlation for survival (10,11). Methods for evaluation of current diagnostic "sanctuaries," such as the retroperitoneum, may therefore be relevant.

At present, the adrenals can be visualized with scintigrams (36). Accumulation of imaging isotope in the glands takes 2–5 days, thereby restricting practical application of the method. Ultrasonically guided fine-needle biopsy could be a useful method, but no data are available. With the use of CT scanning, metastatic involvement of the pancreas, the adrenals, or the retroperitoneal lymph nodes has been observed in small numbers of patients (31,33,37). In most of them, metastases to other distant sites were observed as well. CT scans may, therefore, be useful in the estimation of the tumor volume as well as the pattern of dissemination but seldom change the allocation of patients to classification in either the limited or the extensive stage of disease. At present, no specific recommendations are proposed for the evaluation of the retroperitoneum in staging patients with small cell lung cancer.

RESTAGING

Restaging is essential for evaluation of the effect of therapy, especially for the definition of a disease-free status. Restaging can be performed early in therapy or at the time of discontinuation of treatment, depending on the therapeutic strategy. The number of procedures used for restaging and the frequency with which they are employed may influence response rates and response duration. This explains why duration of survival is often a more reliable measure when alternative treatments are compared. Description of treatment results should be in accordance with World Health Organization (WHO) recommendations (38). It is obvious that differentiation between complete and partial remissions will depend on the number and type of restaging procedures. It is recommended that a re-evaluation should minimally include a repeat of any procedure that initially was abnormal. Persisting lesions should be further evaluated histologically or cytologically. In clinical trials including radiotherapy, the relapse pattern may have special interest because of intended local control of the disease. Restaging directed toward such sites is recommended in relapses after complete remission. To be classified as a long-term disease-free survivor, the patient must remain continuously free of disease after 2 years from initiation of therapy.

Paraneoplastic syndromes, ectopic peptide production, or other biomarkers that have been observed in an individual patient must be re-evaluated and interpreted in relation to the general treatment result. Symptoms caused by ectopic hormone production must be normalized if eradication of the tumor is regarded as complete. Elevated plasma concentrations must decrease to the range observed under disease-free physiologic conditions. However, an adequate control population for definition of this range may not always exist. As an example, the plasma concentration of the hormone calcitonin is increased in 68% of newly diagnosed patients (39) and does not always reach levels of healthy controls even in long-term, disease-free survivors (40). With regard to neurologic paraneoplastic syndromes, data on reversibility are scarce, but complete normalization of a Lambert-Eaton syndrome has been described (41).

Minimally recommended restaging procedures are summarized in table 2. Neither ectopic hormones nor any other kind of biomarkers can at present be recommended for the general staging or restaging of patients with small cell carcinoma, but they should continue to be evaluated in research programs.

PROGNOSTIC FACTORS

The number of patients classified in limited or extensive disease groups depends greatly on the specificity and the number of the staging procedures used. This could explain why some investigators did not find a significant prognostic difference between the two groups (11) while others did (18,42–44) (table 3).

The performance status is a dominating prognostic variable in most studies (42–45). It is a compound variable, however, which depends on both qualitative and quantitative aspects of the disease and on the general health of the patient. This dependency has partly been uncovered in a multivariate analysis by Cohen et al (46). It is currently recommended that the performance status of patients be included in therapeutic trials.

Data on weight loss, sex, and age of patients are recommended in all studies. Univariate analyses of these variables have demonstrated a negative prognostic impact for men 60–70 years old compared to younger individuals (44,45). In another study, however, neither sex nor age had significant prognostic influence (11). A pretreatment weight loss > 2 kg is also a significant adverse symptom (44,47).

Examination of possible prognostic information from pretreatment laboratory values has disclosed that

TABLE 2.—Recommendations for restaging

Site	Procedure	Recommended	If positive initially	If signs/ symptoms
Primary	Chest X-ray	+		
	Fiberoptic bronchoscopy	+		
Mediastinum	Mediastinoscopy*	+		
	Gallium scan		+	
Bone marrow	Biopsy and aspiration	+		
	Bilateral biopsies		+	
	Scintigrams		+	
Liver	Peritoneoscopy and liver biopsy		+	
	Ultrasonography		+	
	CT scan		+	
Lymph nodes and skin	Fine-needle aspiration			+
CNS	CT scans		+	
	Scintigrams		+	
	Lumbar puncture		+	
	Myelograms		+	
Retroperitoneal organs	CT scans		+	+
	Ultrasonography		+	+
	Laparotomy			+

*Whenever possible.

hemoglobin, serum albumin, and LDH all have significant impact (46). Data on the prognostic influence from the microscopic appearance of the small cell carcinoma are conflicting. According to the subclassification proposed by the WHO in 1977, small cell carcinomas can be divided into the subtypes of oat cell, intermediate, and other (48). Results of subtyping are not completely consistent among pathologists (49). This may partly explain why some find a poorer prognosis in patients with the intermediate cell type (50) while others do not (51,52). A major problem may be the inclusion of tumors with large and small cells in the group called intermediate (48). Until these problems have been solved, subclassification is not generally required. It is recommended, however, that all specimens in a trial be reviewed by the same pathologist and that cooperating pathologists make frequent cross references of pathologic material.

The prognostic influence of dissemination to different organs is summarized in table 3. Differences in staging procedures may partly explain the inconsistency of these results.

CONCLUSIONS

No new alternate staging system was proposed, mainly because the current two-stage system is sufficient as long as no stage-specific treatment of the small cell carcinoma is required. New diagnostic methods and accumulating experience with already established methods make it increasingly necessary, however, to evaluate the staging procedures currently available. The lack of specificity of most radionuclide scintigrams is generally recognized, and the clinical role of these diagnostic methods may diminish in the future. Potentially more promising are the new and improved CT scanners and CT-guided or ultrasonically guided fine-needle biopsies. Further evaluation of these staging techniques for patients with small cell lung cancer is encouraged. Apart

TABLE 3.—Prognostic factors

Factor	Correlation with survival*	Ref No.
Limited vs extensive disease	Significant*	(18,42–44)
	Not significant†	(11)
No. of sites	Significant	(11)
Performance status	Significant	(42–46)
Weight loss	Significant	(44,47)
Sex	Significant	(44,45)
	Not significant	(11)
Subclassification	Significant	(50)
	Not significant	(51,52)
CNS metastases	Significant	(11)
	Not significant	(44)
Liver metastases	Significant	(11,44)
Bone metasases	Significant	(44)‡
	Not significant	(11)§

*$P < 0.05$.
†$P > 0.05$.
‡Bilateral iliac crest marrow biopsies and aspiration.
§Bone scan and bone marrow examination.

from diagnostic accuracy, the specificity and sensitivity and the cost and risk of these procedures should be considered. The prognostic impact of any staging procedure should be recognized, but clinical usefulness with regard to an optimal tumor reductive treatment, as well as palliation, should also be evaluated. Although the number of long-term, disease-free survivors has been gradually increasing, it still does not exceed 10%. Nonetheless, complete characterization of such patients with respect to staging and prognostic factors is recommended to determine which factors are consistent with the possibility of achieving long-term survival with current treatment methods. Comparability of data from all institutions will be helpful in the recognition of further improvement in the treatment of this disease. The future will hopefully bring, not only such therapeutic progress, but also general agreement on a practical, useful staging system.

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Role of Radiation Therapy in Small Cell Anaplastic Carcinoma of the Lung

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The previously established roles of radiotherapy in the management of small cell anaplastic carcinoma of the lung have changed with the development of improved chemotherapy. A group of the International Association for the Study of Lung Cancer has concluded that while there is established evidence that the addition of chemotherapy improves the results of radiation therapy, the value of the addition of loco-regional radiation therapy to chemotherapy is uncertain and more difficult to assess. No advantage in short-term (median) survival has been shown in randomized studies, but current data suggest a possible advantage in long-term control of loco-regional disease. Further results of prospective trials are needed to resolve this question. Prophylactic cranial irradiation reduces the incidence of cerebral metastases but so far has not resulted in improved survival. Pending the results of further randomized studies, its routine use in all patients is not advocated. The palliative role of radiation therapy is valuable in symptomatic treatment. Various current modifications of technique to improve the results of conventional radiation treatments with an altered fractionation schedule and use of sensitizers and large fields are not believed to show major advantages. The clear documentation of treatment details and the definition of the criteria for the assessment of results are important if further comparisons of results are to be made possible. In particular, the need for reporting long-term survival at 2 years in contrast to the more usual median survival time is stressed. [Cancer Treat Rep 67:11–19, 1983]

The value of radiation therapy as an effective single treatment modality in the management of small cell carcinoma of the lung has been clearly demonstrated. It has been used with curative intent either in the management of the loco-regional (limited) disease stage or as an adjuvant for occult metastatic disease (1–4). More controversially, it has been proposed as a preoperative adjunct to surgery (5). In addition, it has a major palliative role in the treatment of symptomatic disease. However, recent advances in chemotherapy have made a reappraisal of its role essential.

The First Medical Research Council (MRC) study demonstrated the superiority of radiation therapy over surgery in operable cancers (6). Several randomized studies (7–13) have shown that the addition of chemotherapy to radiation therapy improves the results in limited-stage disease. A summary of the results of the major series is given in table 1.

Improvements in both response rates and survival data with newer chemotherapy must be considered the cornerstone of potentially curative therapy (2,4). The principal question, therefore, is whether or not radiotherapy adds to the results of chemotherapy in the potentially curative management of small cell carcinoma of the lung. This review by the International Association for the Study of Lung Cancer (IASLC) group addresses itself to the problem of the role of radiotherapy in the management of the primary loco-regional disease.

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TABLE 1.—Randomized trials of radiation therapy vs radiation therapy with chemotherapy

Investigator (ref No.)	No. of patients	Median survival (mos)		Chemotherapy
		RT*	RT + CT	
Bergsagel et al (7)	41	5	9.5	CTX
Høst (8)	75	5.5	8.5	CTX
Tucker et al (9)	22	3	6†	CTX
MRC (10)	236	6	9†	CTX, MTX, CCNU
Krauss and Perez (11)	61	5	10‡	CTX, ADR, DTIC
Petrovich et al (12)	59	5	9.5‡	CCNU, hydroxyurea
Mathiessen (13)	34	5	12+†	CTX, ADR, MTX

*RT = radiotherapy, CT = chemotherapy; CTX = cyclophosphamide, MTX = methotrexate, CCNU = lomustine, ADR = doxorubicin, DTIC = dacarbazine.
†$P = < 0.05$.
‡$P = < 0.01$.

its use as an adjuvant in the management of CNS metastases, and specific modifications of its use in attempts to increase its efficacy.

PRESENTATION OF RESULTS

How Should Results Be Reported?

In preparing this report the IASLC group found that a major problem in the comparison of data from various centers has been the variability in the standards of data presentation. Consequently, we include recommendations for the specifications of such details.

Results of treatment.—The assessment of results in terms of survival and local control is hampered by a lack of clarity in specification in current reports. We recommend that, in the future, all groups clearly define these endpoints. In view of the relatively small number of patients entered into most studies and the consequent lack of precision of the median survival time (MST), this particular endpoint is not recommended for comparisons of results, even though we have had to use it in this report because it is all that is usually available. Instead, we believe that reports should contain both crude and actuarial survival times at ≥ 2 years as the principal method of defining treatment results. Details as to whether or not surviving patients are disease-free and the sites of first recurrence will also enhance the possibilities of full interpretation of the data. We believe that an initial analysis of local control should represent a complete response at the loco-regional site as assessed by the conventional specified staging methods.

Specification of radiation dose.—There is a considerable degree of variability in the quality of recording of radiation treatment in various studies and in initial protocols. Minimum requirements for radiation dose specification have been laid down in the World Health Organization handbook (14), but ideally, a more precise specification of treatment should be employed, according to the following criteria.

Treatment volume
- Site
- Field sizes and shapes
- Treatment field plan
- Anatomic structures (regions) included

Treatment source
- Type (isotope or accelerator)
- Beam energy
- Interstitial therapy details

Beam modifiers
- Tissue compensation (physical or calculated)
- Individual field-shaping blocks

Dose specification
- Minimum tumor dose (Gy or rad)
- Maximum critical tissue dose
- Individual fraction size
- No. of treatment fractions
- Overall treatment time
- Continuous or split-course
- Dose rate if not conventional

Time relationship to other treatment modalities

MAJOR TREATMENT QUESTIONS

Does Loco-regional Radiation Therapy Add to the Efficacy of Chemotherapy in Limited Disease?

The data from randomized and nonrandomized studies may be assessed in an attempt to confirm whether or not adding loco-regional radiotherapy improves the quality and duration of tumor control in patients with limited disease. One has to conclude that a definite answer is not yet available.

Collected nonrandomized data suggest that there may be some advantages for the combination of chemotherapy plus radiotherapy over chemotherapy alone. A recent review by Salazar and Creech (3) of the data published in the literature between 1977 and 1979 showed that 33% of 446 patients receiving radiation alone had relapse in the thorax during the course of

TABLE 2.—Nonrandomized studies of chemotherapy with or without radiation therapy

Stage	Therapy	No. of patients	% response		MST range (mos)	% 2-yr disease-free survival
			Complete	Complete + partial		
Limited	CT + RT	492	50	76	6–185	17
	CT alone	246	52	81	5+–14	7
Extensive	CT + RT	734	21	57	3–14	1
	CT alone	990	26	67	4+–14.5	2

their subsequent treatment. The 213 patients receiving chemotherapy alone had an overall relapse rate in the thorax of 82%, whereas in the combination of treatments in 1047 patients, the overall relapse rate was only 28%.

In another recent retrospective review of 2462 patients treated with chemotherapy alone or chemotherapy plus radiotherapy, Bunn and Ihde (15) found improved 2-year, disease-free survival in patients with limited-stage disease receiving the combined-modality therapy (table 2). There were no differences in the overall complete response rates in patients with either limited- or extensive-stage disease, and there were no survival differences within the latter group. However, in both of these studies, the validity of conclusions from such collected data, sometimes with outdated chemotherapy and variable radiotherapy and assessment of endpoints, must be viewed with some caution.

There are now several randomized studies (16–21) of this question and these are tabulated in table 3. No definitive conclusion can yet be reached, and there are contrasting results from the two largest studies. The results of the Copenhagen study (18)[10] show an adverse effect of radiotherapy on survival, but this is not statistically significant. Some authors have speculated that this may be due to more extensive radiation treatment in terms of time/dose relationship than was used in the other series. In the group of patients treated by Fox et al (19)[11] there was an improved 2-year, disease-free survival rate when irradiation was added (one of 44 versus six of 40 patients, $P < 0.025$). This is combined with a reduced relapse rate at the primary site ($P < 0.05$), even though no advantage is seen in terms of the MST. Neither of these two studies included prophylactic cranial irradiation (PCI) in the protocol.

Another study in progress at the National Cancer Institute (NCI) uses concurrent radiation therapy with an intensive chemotherapy regimen (21). Radiation therapy is given with shaped fields that are reduced in size during the fractionated course. Spinal cord and esophageal shielding are used after 24 and 30 Gy, respectively. PCI is given to complete responders. Preliminary results suggest an advantage for the combined-modality treatment (20).

The Southeastern Oncology Group has entered over 250 patients with limited-stage disease into a randomized study investigating the value of the addition of regional radiotherapy to combination chemotherapy (cyclophosphamide, doxorubicin, and vincristine) with PCI. A preliminary report on 65 patients who have completed induction therapy gives a higher total response rate (67% versus 84%) for the combined-modality group (22). Further details of this and other randomized studies of chemotherapy versus chemotherapy with radiotherapy are required, with full assessment of both early and late results.

Is There an Optimum Time Relationship Between Chemotherapy and Radiotherapy?

A further unanswered question in the combined-modality treatment is whether chemotherapy should be delayed until after radiotherapy, given synchronously, or given first. The theoretic advantages of simultaneous treatment are that the chemotherapy may enhance the radiation effect on the primary tumor and that there will be immediate control of both primary and secondary disease by avoiding long periods without chemotherapy. Disadvantages include increased toxicity and difficulty in assessing the response to chemotherapy because of the effect of concurrent radiotherapy.

Consecutive treatments have the advantages that they avoid overlapping toxic effects and that an assessment of the chemotherapy response can be made first. The disadvantage is protraction of treatment. The advantage of using radiation therapy first is that it directs known effective therapy to the primary and usually symptomatic site. However, this does delay the employment of chemotherapy, which may permit further extension of untreated systemic disease.

There are no unequivocal data that resolve this question, which is discussed with full references in two recent reviews (1,3). Indirect evidence from several studies supports all the sequence concepts. The value of treatment with either drugs or radiation commenced first has been investigated in two randomized studies. The Swiss Group for Clinical Cancer Research found no sig-

[10]Results updated by P. Dombernowsky in a personal communication, Sept 1981.

[11]Results updated by R. M. Fox in a personal communication, Sept 1981.

TABLE 3.—Randomized trials of chemotherapy vs chemotherapy plus radiotherapy*

Investigator (ref No.)	Treatment†	No. of patients	MST (mos)		2-yr disease-free survival‡			
					Projected %		Actual	
			CT	CT + RT	CT	CT + RT	CT	CT + RT
Completed studies								
Williams et al (16)	CTX, VCR, CCNU, PCB 30 Gy/2 wks§ All PCI	25	11	9	30	0	1/12	0/13
Stevens et al (17)	CTX, VCR, ADR 35 Gy split§ All PCI	32	12	11.5	NA	NA	NA	NA
Dombernowsky et al (18) and footnote 10¶	CTX, VCR, CCNU, MTX 40 Gy/split/2 wks§ No PCI	154	12	10.5	NA	NA	13%	8%
Ongoing studies								
Fox et al (19) and footnote 11**	CTX, VCR, ADR 40 Gy/4 wks§ No PCI	84	14.5	14.5	NA	NA	1/44	6/40
Cohen et al (20,21, updated personal communication)††	CTX, MTX, CCNU alternating with VCR, ADR, PCB No PCI	37	14	14	15	40	1/9	4/10

*All series had patients with limited-stage disease except those in ref 16 with extensive-stage disease.
†PCB = procarbazine; *see* table 1 for list of other drug abbreviations.
‡NA = data not available.
§Thoracic RT.
¶Local recurrence reduced in CT + RT group (48%) vs CT only (78%). Actual results at $2^1/_2$ yrs.
**2-yr disease-free survival ($P < 0.05$). Initial relapse at primary site CT only 24/44 vs CT + RT 14/40 ($P < 0.05$).
††CT alone, CR 10/18 (56%) vs CT + RT, 13/19 (68%).

nificant difference in a study in which the relevant trial subgroups were small (23 and P. Alberto, personal communication, 1981). The third MRC Small Cell Study has been investigating this same problem. Case accrual has closed, but the patient follow-up continues, and definitive results are not yet available. The Cancer and Leukemia Group B investigators are currently studying the important question of simultaneous versus consecutive treatment with chemotherapy and radiotherapy, but this study is in progress, and no results are yet available.

Does Loco-regional Radiation Therapy Have a Role in Extensive Disease?

There is a paucity of data on whether radiation treatment has any different role in the management of loco-regional disease in extensive disease than in limited disease. In a small study of 25 patients by Williams et al (16), which compared chemotherapy plus PCI with or without loco-regional radiation therapy, no differences in MST or sites of relapse were found. In a larger study by the Working Party in Lung Cancer (protocol WP-L 72-22), 118 evaluated patients were randomized to receive a three-drug regimen together with PCI (30 Gy), abdominal irradiation (20 Gy), and chest irradiation (20 Gy) or to receive chemotherapy alone. There was no survival advantage seen for the more intensive treatment (24).

Therefore, the role of radiotherapy in patients with extensive disease remains to be clarified: given the inability of current chemotherapy to control disease in multiple sites beyond the chest, it is unlikely that the addition of radiotherapy will improve survival. However, there may be a set of patients with life-threatening intrathoracic tumors; palliative irradiation may be indicated and it may prolong survival. As the efficacy of chemotherapy increases, radiotherapeutic control of bulky intrathoracic tumors may become more important.

What Is the Correct Volume for Loco-regional Radiation Therapy?

Tolerance of the total radiation dose is limited by certain critical normal tissues such as lung, spinal cord, heart, and esophagus. Excessive treatment of these tissues may result in either short-term discomfort to the patient or risk of long-term morbidity and even mortality. The planning technique of radiation therapy is very important.

The IASLC group believed that precision in selective shielding of an uninvolved lung, using shaped protective blocks as already used in the radiation treatment of lymphomas, provides the best technique for reducing unwanted lung damage. The radiation therapy planning will be helped by computed tomography, which usually defines with precision the tumor volume and also reveals unexpected spread, for instance, along the paravertebral gutter.

The minimum volume for treatment should be defined with precision, and this should be reduced during the radiation course to match tumor shrinkage. However, it remains an open question whether the volume treated with radiation should relate to the tumor seen after initial chemotherapy or the volume present during the initial prechemotherapy staging procedures. There are no data that answer this question.

Several participants in the group believed that the use of a posterior block for shielding both the spinal cord and the esophagus after 20–30 Gy is important to permit adequate radiation dose. However, others believed that attempts to shield these particular organs, especially the esophagus, with a posterior block only results in tumor shielding and did not recommend it. The tolerance of these critical normal tissues depends on the total radiation dose and may be influenced by associated chemotherapy. Therefore, no firm recommendations are made, other than to be aware of the potential risks.

What Radiation Dose Is Required for Loco-regional Disease?

The radiation dose dependence of local tumor control has not been tested in a randomized study. All the data available from the many studies in which different doses have been employed are circumstantial. These studies have been reviewed elsewhere (1,3,25).

All studies, over a wide range of radiation dose (25–60 Gy with varying fractionation but usually 2–3 Gy/day), show a significant percentage of failure within the radiated volume in the chest. Data are frequently incomplete, with short follow-up, but they have been cited to range from 9% on the basis of clinical evidence (3) to 69% at autopsy (25) for patients receiving radiation therapy alone.

There is evidence to suggest that when irradiation is combined with chemotherapy, the radiation dose may be reduced. Thus, as in a retrospective survey by Cox et al (26), the median dose required to produce control of the thoracic tumor was less with the combined-modality treatment than with radiation alone. It is likely that this dose of radiation will be dependent on the nature of the chemotherapy regimen given and may be unique for that particular combination of drugs.

In current practice the dosage given to the loco-regional disease ranges between 30 Gy over 2 weeks to 50 Gy in 5 weeks, given as five fractions a week. Other physicians, however, favor a more concentrated radiation dose given as a split course. There is no evidence that this results in any better survival, but it is thought

by some to be better tolerated by the patients. The group believed that small cell carcinoma needs the same radiation dose as other epithelial tumors for long-term control, but there are indications that chemotherapy may modify this dose response (26).

Does Prophylactic Cranial Irradiation Add to the Efficacy of Chemotherapy With or Without Locoregional Radiotherapy?

The exact role of prophylactic or elective cranial irradiation for this common site of metastasis has not been resolved. A compilation of results from nonrandomized trials, which employed PCI in a variety of dose and fractionation schedules, has demonstrated a reduction in the CNS relapse rate from 22% in patients not given PCI to 8% in those receiving PCI (27). There is no obvious beneficial effect of PCI on MST in these series. Results from seven randomized trials studying the effects of PCI have been published to date and are shown in table 4 (28–34). Five of these trials have shown a significant reduction in the CNS relapse rate, while two have shown no effect. None of the trials has shown a survival benefit for PCI. It is difficult to ascertain whether the dose or timing of PCI is important, particularly since the highest and lowest PCI doses were in the negative trials and since PCI was given in one of these trials on Day 1 and in the other at Week 12.

Definitive conclusions from these studies are also difficult because crude rather than actual or actuarial relapse percentages are reported, follow-up periods are short, autopsy data are not provided, and the influence of systemic response to treatment is not considered. Only the small trial of Aisner et al (34) has evaluated the rate of primary relapse from complete remission, and there are no randomized data with respect to survival of complete responders with and without PCI. A recent review of a large, nonrandomized 10-year experience with and without PCI at the NCI (35) has demonstrated that patients who do not achieve a complete response (CR) have a very high likelihood of developing CNS relapse (approaching 100% for patients surviving ≥ 2 years) whether or not PCI is given. Since these patients also have short survival (≥ 90% die within 1 year and ≥ 99% within 3 years), generally have relief of CNS symptoms from therapeutic brain irradiation, and usually are symptomatic from other sites of metastatic disease, it was the consensus of this group that PCI need *not* be given to patients with extensive disease who fail to achieve a CR.

In the NCI experience, patients with CR who were given PCI had significantly fewer CNS relapses, although this difference was no longer significant when stage of disease and liver involvement (the two most important CNS risk factors by multivariate analysis) were considered (35). And while the 2- and 3-year disease-free survival rates were higher in patients with CR who were given PCI, the survival curves were not significantly different. Primary CNS relapse was observed in eight of 48 patients with CR who were not given PCI but in none of 75 patients with CR who were given PCI. The IASLC group could not reach a consensus on the role of PCI in complete responders. Some felt the current data suggest that all patients with CR should receive PCI, some felt that no patients with CR should receive PCI, but most felt the issue was unresolved. The IASLC group therefore strongly recommended that randomized prospective trials stratified by disease stage and liver involvement should be undertaken. The group felt that when PCI is employed, the most frequently prescribed dose schedules range from 2400 rad in ten fractions every 2 weeks to 3000 rad in

TABLE 4.—Randomized trials of PCI

Investigator (ref No.)	No. of patients	PCI dose (cGy)	PCI time	% patients with CNS relapse			Survival significance*
				PCI	No PCI	*P* value	
Jackson et al (28)	29	3000	Day 1	0	27	< 0.05	NS
Maurer et al (29)	163	3000	Weeks 8–12	4	18	< 0.009	NS
Hirsch et al (30)	111†	4000	Week 12	9	13	NS	NS
Beiler et al (31)	54	2400	Week 3	0	16	< 0.05	NS
Cox et al (32)	45	2000	Day 1	17	24	NS	NS
Seydel et al (33)	271	3000	Day 1	5	21	< 0.05	NS
Aisner et al (34)	29‡	3000	At CR	0	36	< 0.02	NR
Total	702			6	20		

*NS = not significant, NR = not reported.
†Limited-stage disease alone studied.
‡Complete responders alone studied.

15 fractions every 3 weeks and that PCI can be given any time a CR is achieved between 4 and 20 weeks.

The group also felt that decisions regarding the best approach to CNS metastases would be aided by additional information in several areas.

(*a*) There are very few data on the primary CNS relapse rate with and without PCI when primary CNS relapse is defined as the sole site of relapse from CR. Excluding the references cited above, primary CNS relapse was reported in seven of 241 patients (3%) given PCI (36,37). No other data for patients not given PCI could be found.

(*b*) There are very few data regarding therapeutic irradiation of established metastases, either at presentation or relapse. Two published trials have demonstrated near total relief of symptoms in 64% and 92% of patients who received a full course of cranial irradiation (38,39). The number of patients who die from CNS metastases before receiving a full course of irradiation, the number who relapse after cranial irradiation, and the most optimal dose of therapeutic irradiation are unknown. In another study evaluating all patients who developed brain metastases, whether they completed radiotherapy or not, 45% subsequently died from their brain metastases, a failure rate greater than that seen following PCI (40).

(*c*) There are few data on the acute and chronic effects of PCI, particularly with respect to neurologic, visual, and auditory functions. The only report to date has shown CT abnormalities but no clinical or EEG changes following PCI (41).

(*d*) Current data suggest no effective means for the early diagnosis, prevention, or therapy of leptomeningeal, spinal, or epidural metastases (42). Hopefully, trials providing new information in these areas will be forthcoming in the near future.

OTHER TREATMENT QUESTIONS

Several specific additional problems were considered by the group.

How Should Superior Vena Caval Obstruction Be Treated?

It is believed that both irradiation and combination chemotherapy are effective in previously untreated patients with superior vena caval obstruction at presentation, and one probably does not have any particular advantage over the other. Treatment usually depends on the local practice or on the specifications of the protocol. The group's impressions of the response rates and speed of response appear to be similar for both treatments, although few hard data are available. It was felt that there is usually no excuse for not establishing a diagnosis in such patients prior to therapy, as most will remain in a stable condition with supportive therapy such as steroids until the diagnosis is made. Radiation therapy is the treatment of choice in patients developing superior vena caval obstruction at relapse whether or not it was the initial treatment.

Is There a Role for Extrathoracic Radiation As Part of Primary Treatment?

Initial studies have so far been unrewarding, as the study reported by Williams et al (16). It was thought that this was not likely to be a very useful general approach to the disease but that it might be suitable for a limited specialist group study.

Is There a Role for Total Body or Hemi-body Irradiation?

Irradiation of either the whole or upper and lower halves of the body with large radiation field and small doses has received some attention over recent years, mainly as a palliative measure. The major problem is toxicity, particularly when irradiation is combined with chemotherapy. In limited series of patients studied by several groups (43–48), tumor responses have been seen, but they have been relatively small and unsustained. In one randomized study of 64 patients, total response rates were similar for upper and lower hemi-body irradiation and for a triple-drug regimen. All patients received loco-regional radiotherapy. The estimated MST was the same for patients with limited disease but was much worse for those with extensive disease who had hemi-body irradiation (48). No details of long-term survival are available. Other details are summarized in table 5. The conclusion, therefore, must be that this large-field irradiation technique does not appear to be especially promising in the management of small cell lung cancer. However, it is recognized that a technique employing one or two radiation treatments giving results similar to those of an extensive course of treatment by combination chemotherapy would have advantages, provided that this was justified by an assessment of results over a sufficient time. Techniques involving bone marrow reconstitution may also have an advantage with such treatments.

Will Other Radiation Techniques Be of Value?

Other types of radiation such as neutrons, negative pi-mesons, and other high linear energy transfer radiation are unlikely to help. The possible increase in local control would not begin to show any effect in terms of survival until there have been considerable improve-

TABLE 5.—Role of large-field radiation therapy*

Investigator (ref No.)	No. of patients	Type of irradiation	Dose/fraction (Gy/No. of fractions)	Other treatments	Results
Monaghan (43)	6	TBI	1.5/10	Local RT	MST 20 wks
Dawes (44)	22	TBI	1.5/1 in 10	None	MST 20 wks Radical
		HBI × 2	6–8/1	None	MST 10 wks Metastatic disease
Salazar et al (45)	19	HBI (upper)	6/1	Local RT + CT	Questionable improvement in retrospective controls
Woods et al (46)	29	HBI × 2	7/1	CT × 2 before RT	MST 28 wks Poor prognosis
Byhardt et al (47), randomized	8 7	TBI None	1/10 —	Local RT Local RT + CT	No patients alive 1 alive at 19 mos
Urtasun et al (48), randomized	30 34	HBI × 2 None	8/1 —	Local RT Local RT + CT	87% response rate† 88% response rate

*TBI = total-body irradiation, HBI = hemi-body irradiation.
†Estimated MST for limited disease: HBI, 43 wks; CT, 42 wks. Estimated MST for extensive disease: HBI, 15 wks; CT, 44 wks. Estimated MST for complete responders: HBI, 51 wks; CT, 62 wks.

ments in chemotherapy. The group believed that the same is likely to apply to the use of radiation sensitizers.

CONCLUSIONS

The group concluded that although radiation treatment was a valuable adjunct in the management of small cell carcinoma of the lung, it no longer plays a primary role. The exact definition of its value in curative management remains to be assessed, and a definitive answer will need to await more effective chemotherapy than is currently available. Modifications in radiation treatments should probably follow sequentially. Early assessment of trials on the basis of MST may result in misleading conclusions, and it is essential that long-term control as defined by survival, 2-year survival, or disease-free status should always be employed.

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Complications of Treatment of Small Cell Carcinoma of the Lung

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Improvements in therapy for small cell carcinoma of the lung have been achieved with treatment regimens that result in significant toxicity. The workshop of the International Association for the Study of Lung Cancer focused on the following complications of chemotherapy and radiotherapy: leukopenia and resultant infections, esophagitis, pneumonitis and pulmonary fibrosis, cardiac toxicity, second malignancies, neurologic complications, and psychosocial effects. The data regarding the incidence and management of these complications are briefly reviewed, and recommendations for further studies are noted.
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The International Association for the Study of Lung Cancer workshop on small cell carcinoma of the lung clearly documents that developing better therapeutic approaches and achieving more complete remissions must be the top priorities in clinical research in this type of lung cancer. However, the assessment of the complications of current therapies is important in determining the risk-benefit ratio for our patients and in developing new therapeutic strategies. This summary of the session on the complications of therapy has the goal of stimulating improved recognition and better reporting of complications. This paper is not intended to provide an exhaustive review of the available literature but to highlight key areas for further investigation.

Before discussing the therapeutic complications, the working group felt it was important to focus on the patient's pretherapy medical status. Patients with small cell carcinoma of the lung are generally in the fifth to seventh decades of life and often have other significant medical problems such as chronic obstructive pulmonary disease, arteriosclerotic cardiovascular disease, benign prostatic hypertrophy, and peptic ulcer disease. These medical problems certainly can impact on the development of complications related to antitumor therapy. A performance status categorization more detailed and specific for the patient with small cell carcinoma than the standard Eastern Cooperative Oncology Group or Karnofsky scale could perhaps define risk factors for the subsequent development of complications. This would be particularly important in terms of the status of pulmonary symptoms where scales of respiratory symptoms are available (1). Objective measurements of pulmonary function such as spirometry and measurement of the single-breath diffusing capacity could also be performed prior to therapy and repeated at regular intervals during and following cytotoxic treatment.

Discussion of the complications of therapy is broken down into recognized and potential complications, although this is obviously an arbitrary division. Virtually all of the toxic effects can be caused by either chemotherapy or radiotherapy alone, and combined-modality therapy certainly increases the risk of such problems. Only major complications of therapy are discussed. Toxic effects such as nausea and vomiting and alopecia are obviously of great concern to the patient but are not the subject of this report.

RECOGNIZED COMPLICATIONS

Leukopenia and Resultant Infections

Most chemotherapy regimens used for the treatment of small cell bronchogenic carcinoma are associated with some degree of myelosuppression. Radiation of the in-

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trathoracic region and skull can affect 20%–25% of functioning bone marrow (2), so radiation therapy to the primary tumor and prophylactic cranial radiotherapy also contribute to myelosuppression.

It is extremely difficult to make statements about the frequency of granulocytopenia and related infectious complications in these patients, since the degree of marrow suppression varies a great deal according to the type of chemotherapy used. With most reported standard chemotherapy regimens used for the treatment of small cell carcinoma, the duration of granulocytopenia has been relatively short; febrile episodes are reported in about 30% of the treated patients, documented infections in about 5%, and fatal infections in 2%. When chemotherapy is combined with radiotherapy, the rate of infectious complications is somewhat higher at 11.7%, and fatal infections have been reported in 2.7% of the cases (3).

However, when more intensive chemotherapy is utilized in an attempt to achieve higher complete response rates and longer survival times, granulocytopenia is more severe and the infectious complications are more frequent. Meticulous attention to the therapy and prevention of these complications is essential in conducting this type of therapeutic research. In these research trials, it is also important to fully report the hematologic and infectious complications with descriptions of nadirs of blood counts, duration of cytopenias, number of febrile episodes and documented infections, and microbiologic details of infectious complications. Special problems such as infections that occur when patients are not leukopenic and pulmonary complications (obstructive pneumonia and lung abscess) must also be reported.

Treatment of Infections in Neutropenic Patients

Granulocytopenia predisposes to a high frequency of bacterial infection and also to bacterial and nonbacterial superinfection. Infection can be demonstrated in about 40% of febrile neutropenic patients; in 50% of them, bacteremia can be documented; and in another 20%, no specific bacterial pathogens can be demonstrated, but the presence of infection is presumed on the basis of clinical signs and symptoms. Thus, it can be safely estimated that a febrile episode is associated with infection in at least 60% of neutropenic patients. These observations provide justification for the use of empiric antimicrobial therapy in febrile neutropenic patients (4).

The next important question relates to the type of antibiotics that should be used in febrile neutropenic patients. These antibiotics should provide adequate action against the most frequently isolated pathogens: *Escherichia coli*, *Klebsiella* sp, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. These four pathogens are the causative micro-organisms in about 80% of the bacteremic episodes in neutropenic patients. No single antibiotic has been demonstrated in the clinical setting of neutropenia to have a spectrum broad enough to act against all these pathogens.

The recognition of the difficulty in diagnosing infection in the neutropenic host as well as an increasing awareness of the relationship between the granulocyte count and risk of infection have resulted in improved survival during induction therapy for acute myelocytic leukemia through the early use of broad-spectrum antibiotics during episodes of severe granulocytopenia. These principles have also been applied with good results in the intensive therapy of patients with small cell carcinoma. For example, at The Johns Hopkins Oncology Center, high-dose induction therapy with cyclophosphamide, doxorubicin, and etoposide was utilized in a population of 54 patients with localized and extensive small cell carcinoma (5). This induction therapy resulted in a median wbc count nadir of 121/mm^3. The wbc count remained $< 1000/mm^3$ for an average of 8 days, and there were 82 febrile episodes in the 104 courses of intensive chemotherapy. Systemic antibiotic therapy with gentamicin and carbenicillin or trimethoprim-sulfamethoxazole and carbenicillin was routinely used when leukopenic (wbc count $< 1000/mm^3$) patients became febrile. There were no deaths due to infection in leukopenic patients. There were three treatment-related deaths (5.5%), and all were due to pulmonary complications.

Patients who respond to empiric therapy with antimicrobial drugs but remain neutropenic should be treated with the initial empiric regimen as long as the neutropenia persists. The mortality rate seems to be lower in the patients who continue to receive antibiotics compared to those in whom antibiotic therapy is discontinued sooner.

In addition to improving diagnostic and therapeutic approaches to bacterial infection, attention must be directed to viral infections as complications of more aggressive and prolonged cytotoxic regimens. In reports from two research units, herpes zoster has been diagnosed in 8%–12% of patients with small cell carcinoma treated with chemotherapy with and without radiotherapy (6,7). Approximately one-half of the cases of herpes zoster occurred during complete remission. The viral infections were diagnosed 6–25 months after the start of antitumor therapy. With the advent of improved treatment of viral infections (8), better documentation of these infections in patients with small cell carcinoma is urged.

Prophylactic Measures

Prevention of infections in patients receiving intensive therapy for hematologic malignancies and solid tumors is receiving increasing attention. Since most

bacterial infections in cancer patients are caused by pathogens in the gastrointestinal tract, which has been colonized by resistant hospital organisms, the prophylactic use of oral, nonabsorbable antibiotics is a logical approach to the prevention of these infections.

A recent study by the Antimicrobial Therapy Project Group of the European Organization for Research on Treatment of Cancer (EORTC) has indicated that patients who have received some form of gastrointestinal decontamination had a significantly lower incidence of severe infection compared to patients who have not received such prophylaxis.[9] Many regimens have been proposed for gastrointestinal decontamination of neutropenic patients. These include aminoglycosides, polymyxins, and co-trimoxazole. Evidence has recently been presented suggesting that co-trimoxazole may be of benefit in decreasing infectious complications during chemotherapy of small cell carcinoma (9). However, the important factor that greatly influences the efficacy of these regimens is the pattern of resistance of the microbiologic flora in the particular hospitals where these regimens are applied. These differences can probably explain the contradictory results published by various investigators. Nevertheless, it is likely that the combination of orally administered antibiotics (provided they are effective against most pathogens found in the hospital environment) and antifungal agents is effective prophylaxis against gram-negative bacillary infection in neutropenic patients.

Lithium, which has been shown in psychiatric patients to produce a rise of the granulocyte count, has been used prophylactically against severe infections in patients receiving chemotherapy for small cell cancer. Severe episodes of infection were significantly reduced in patients receiving lithium in a prospective controlled study (10). However, these results must be confirmed by other controlled trials. In addition, the utility of lithium administration in patients with prolonged granulocytopenia remains to be determined.

Esophagitis

Mild esophagitis can occur in a considerable number of patients receiving mediastinal irradiation. However, in the management of patients with small cell carcinoma of the lung, the greatest concern is the esophageal toxicity that has been reported in regimens utilizing both chest radiotherapy and doxorubicin chemotherapy. Great concern was caused by the study of Brereton et al (11) at the Radiation Oncology Branch of the National Cancer Institute (Bethesda, MD), in which intensive chemotherapy was used concomitantly with high doses of radiation therapy administered in multiple schedules. In this study, > 50% of the patients developed moderate to severe esophagitis and 5.6% developed esophageal strictures. This has led to the recommendation that concomitant use of doxorubicin-containing regimens and thoracic radiotherapy should be discouraged so that serious and potentially fatal esophageal toxicity can be prevented (3). However, subsequent reports have emphasized the importance, not only of the timing of radiation therapy and chemotherapy, but also of the volume of the area treated and the dose rate of both types of cytotoxic therapy. With the use of modern radiotherapy planning, several groups have now reported the concomitant use of doxorubicin-containing chemotherapy regimens and thoracic radiation therapy without a high incidence of severe esophageal complications (12,13). This problem requires further investigation. It is extremely important that severe cases of esophageal toxicity are reported in therapeutic studies of small cell carcinoma.

Pneumonitis and Pulmonary Fibrosis

Radiation therapy is generally the major cause of treatment-related acute pneumonitis and chronic fibrosis in patients with lung cancer, but cytotoxic drugs can also add to this problem. Radiation pneumonitis and fibrosis are related to the volume of irradiated lung, total dose, and fractionation. Except in the studies of Brereton et al (11) and the protocols in which bleomycin was used in conjunction with radiation therapy (14), radiation pneumonitis has not been a common complication of treatment of small cell carcinoma. However, as treatment programs become more intense and complicated, precise reporting of acute pulmonary problems is essential. In addition, careful monitoring of long-term survivors for problems such as pulmonary fibrosis is warranted. In a review of long-term survivors at the NCI-Veterans Administration Hospital, Bunn et al (15) noted that seven of 16 patients who were alive without relapse for a period > 30 months had pulmonary fibrosis. Serial studies of pulmonary function (eg, spirometry and single-breath diffusing capacity) and use of standard classification of pulmonary function prior to, during, and following therapy for small cell carcinoma are strongly recommended.

Cardiac Toxicity

Cardiac complications are recognized problems in combined-modality therapy used in Hodgkin's disease. However, cardiotoxicity has been a relatively rare complication in the treatment of small cell carcinoma. The types of cardiotoxicity that could, however, be anticipated in this group of patients include pericarditis, aggravation of coronary artery disease, and cardiomy-

[9]EORTC International Antimicrobial Therapy Project Group. Amikacin and carbenicillin with or without cefazolin as empirical treatment of febrile neutropenic patients. Submitted to *Lancet*.

opathies particularly related to doxorubicin. The use of modern radiologic techniques such as gated blood pool scans should prove useful in the assessment of cardiac toxicity in therapeutic approaches in small cell cancer.

POTENTIAL COMPLICATIONS

Obviously, complications could occur in any organ system, depending on the specific therapy involved. However, the working group focused on three areas of particular concern.

Second Malignancies

The advent of combined-modality therapy has resulted in the prolongation of survival and the cure of a significant number of patients with advanced cancer. The enthusiasm for even more intensive therapy has been tempered by the recognition of long-term sequelae such as second malignancies, particularly acute leukemia.

While long-term survival is not achieved by the majority of patients with small cell carcinoma of the lung, approximately 10%–20% of these patients will survive free of disease for 2 years following the initiation of treatment. As noted in table 1, four cases of acute leukemia that developed after therapy of small cell carcinoma are now reported in the literature (16–18). There are not enough data to even attempt to define an incidence figure for the development of acute leukemia. However, the cases reported appear to follow a pattern observed in Hodgkin's disease, multiple myeloma, and ovarian carcinoma where leukemia has occurred several years after the initiation of irradiation and chemotherapy. The four patients with small cell carcinoma (table 1) had achieved a complete remission, and the leukemia developed from $2^1/_2$ to 3 years following the diagnosis of lung cancer. Two of the patients had an erythroleukemia and all patients had an accompanying sideroblastic anemia. Three of the four patients received combined radiotherapy and chemotherapy. All of the patients had prolonged therapy for a minimum of 22 months and received both cyclophosphamide and a nitrosourea. Three of the four patients were treated with procarbazine, and three had chromosomal abnormalities including hypodiploidy and chromosomal rearrangements.

There are even fewer data regarding the development of other solid tumors, including other types of lung cancer, in this population of patients. Obviously, it is important to document such cases in the literature. In the patients with small cell carcinoma who achieve complete remission and long-term survival, thorough reevaluations are indicated at the time of relapse. Repeat biopsy of suspicious lesions for morphologic as well as biochemical and tissue culture studies is recommended.

Neurologic Complications

Small cell carcinoma can cause myriad neurologic complications as a result of direct as well as indirect effects of tumor. Direct effects of masses can include intracranial metastases, leptomeningeal carcinomatosis, and spinal cord compression. Indirect effects can include cognitive defects, limbic encephalopathy, Eaton-Lambert syndrome, and peripheral neuropathies. In this setting, it can be difficult to recognize neurologic complications of the therapy for small cell carcinoma. Such potential complications include peripheral neuropathy secondary to chemotherapy and radiation-induced transverse myelitis.

TABLE 1.—Acute leukemia after therapy for SCC*

Patient No.	Age/sex	Stage of SCC	Therapy and duration (ref No.)	Response of SCC	Time to leukemia	Type of leukemia†
1	63/F	Extensive: marrow involvement	CAV-BOMP, 2 yrs Radiotherapy (16)	CR	3 yrs	Acute nonlymphocytic
2	58/F	Localized	CAV-BOMP, 2 yrs Radiotherapy (16)	CR	$2^1/_2$ yrs	Erythroleukemia
3	65/M	Extensive: nodal involvement	CCM,‡ 22 mos (17)	CR	2 yrs 10 mos	Erythroleukemia
4	59/M	Extensive: nodal involvement	CCM, 23 mos Radiotherapy (18)	CR	3 yrs	Acute myelocytic

*SCC = small cell cancer; CAV = cyclophosphamide, doxorubicin, and etoposide; BOMP = carmustine, vincristine, methotrexate, and procarbazine; CCM = cyclophosphamide, lomustine, and methotrexate.

†All 4 patients had sideroblastic anemia.

‡Doxorubicin, vincristine, and procarbazine at relapse.

The potential complication that was of greatest concern to the working group was the adverse effect of prophylactic cranial radiotherapy. With the exception of the study of Brereton et al (11), there has been little mention of adverse effects of prophylactic cranial radiotherapy in the population of patients with small cell carcinoma. In that study, 42% of the patients who received 3000 rads of prophylactic cranial radiation therapy in 200-rad fractions in conjunction with thoracic radiation and systemic chemotherapy developed a syndrome that included memory loss, tremor, dysarthria, slurred speech, somnolence, and myoclonus. This syndrome began within 3 months of initiation of prophylactic cranial radiotherapy and resolved during the ensuing 3–6 months without apparent residual effects.

In children with acute lymphocytic leukemia who have received prophylactic cranial radiotherapy, abnormalities of computed tomography (CT) scans of the brain, abnormalities in hypothalamic-pituitary function, and recently, impairment in intellectual and perceptual motor functions have been reported (19). In the elderly group of patients with small cell carcinoma, vigilance regarding the development of neurologic deficits due to modalities such as prophylactic cranial radiotherapy is certainly justified. Catane et al (20) have studied a group of 13 long-term survivors with small cell carcinoma who had been treated with prophylactic cranial radiotherapy. Although CT abnormalities were detectable, no significant clinical abnormalities were noted in this small group of patients. However, studies focusing on thorough pretherapeutic neurologic evaluation and serial studies of mental status, CT scans, and electroencephalograms in patients receiving prophylactic cranial radiotherapy are recommended.

Psychosocial Effects

The working group felt that it certainly was not too early in the evolution of therapeutic studies in small cell carcinoma to begin to focus on the psychologic, social, and economic consequences of the diagnosis and treatment of this very common malignancy. Such studies should focus particularly on the specific psychosocial problems to be expected in this group of elderly patients and should concentrate on better means of supporting them during the cytotoxic therapy.

CONCLUSION

This discussion of recognized and potential complications of the therapy of small cell carcinoma is not meant to be all-inclusive but only to focus on major areas of concern. As new therapeutic approaches are utilized, such as autologous marrow rescue, hemibody radiation therapy, immunotherapy, and special nutritional techniques, even more attention will have to be paid to the complications of treatment. It is the hope of this working group that in the near future, there will be many more long-term survivors and considerably more precise data regarding the complications of the increasingly successful therapy.

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Experimental Studies of the Biology of Human Small Cell Lung Cancer

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Small cell lung cancer (SCLC) accounts for 20%–25% of all cases of primary lung cancer and differs clinically and biologically from the other three varieties of lung cancer: squamous cell lung cancer, adenocarcinoma, and large cell lung cancer (the so-called non-SCLC tumors). These differences include: (*a*) more rapid tumor cell proliferation (1,2), (*b*) greater tendency for early regional and systemic dissemination (3), and (*c*) greater sensitivity to both chemotherapy and radiation therapy (4). Although 70%–95% of patients with SCLC initially respond to cytotoxic therapy, only 5%–10% are cured. In most studies, both tumor response to cytotoxic therapy and survival are related to extent of disease and performance status of the patient at diagnosis.

The origin of SCLC is still uncertain. Initially it was considered to be an undifferentiated tumor associated with a variety of paraneoplastic syndromes caused by "ectopic" hormone secretion. In the late 1960s, the recognition that these tumor cells contained neurosecretory granules (5,6) led to their inclusion in the amine precursor uptake and decarboxylation (APUD) cell system of Pearse (7). Thus, it was presumed that SCLC arose from endocrine cells (Kulchitsky's cells) in the normal respiratory mucosa, which expressed APUD properties (8).

To further expand our understanding of the origin and biology of SCLC, in vitro and in vivo experimental systems have been developed that permit the continual in vitro growth and characterization of tumor cells obtained from patients with SCLC and that have greatly expanded our knowledge concerning the origin and biology of SCLC, with important clinical ramifications. The laboratory approaches used in studying SCLC can be subdivided into the following:

(*a*) in vitro culture, establishment, and characterization of human cell lines of SCLC;

(*b*) development of an in vitro clonogenic assay for clinical specimens of SCLC, which will permit in vitro drug sensitivity studies;

(*c*) identification of specific growth requirements of SCLC, using both established cell lines and fresh clinical specimens; and

(*d*) use of athymic nude mice for the characterization of clinical specimens and cell lines of SCLC.

The in vitro growth of cell lines of SCLC has permitted the identification of biologic products associated with SCLC, the generation of monoclonal antibodies with specificity for SCLC, and the identification of a specific cytogenetic abnormality found thus far only in tumor cells of small cell origin. All of these factors have direct application to patient treatment.

IN VITRO CHARACTERISTICS OF CONTINUOUS CELL LINES

Over the past several years, investigators from different laboratories have successfully cultured, in vitro, many cell lines of SCLC (9–12). These cell lines have been cultured from newly diagnosed patients and from patients who have relapsed from prior therapy. In addition, cell lines have been successfully established from autopsy specimens (10,11). In most instances, cell lines were initiated from metastatic specimens of SCLC, including bone marrow aspirates and biopsies, lymph node aspirates and biopsies, biopsies of metastatic cutaneous nodules, and malignant pleural effusions. A small number of cell lines have been established from primary lung masses and liver and brain metastases (9–12). Two approaches have been used to initiate and culture fresh clinical SCLC specimens: (*a*) the direct culture in liquid medium using either a nonselective, serum-supplemented medium or a chemically defined medium selec-

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tive for SCLC; and (*b*) the inoculation of fresh specimens into nude mice with subsequent culture of heterotransplants in liquid medium. While initial attempts to culture these cells were generally unsuccessful, with improved techniques, experience, and growth conditions, cell lines can be successfully established from most specimens containing SCLC tumor cells. Initial attempts to culture SCLC used a variety of different growth media supplemented with 10%–20% heat-inactivated fetal bovine serum. Most cell lines have been established in either RPMI-1640 or Waymouth's MB-7521 medium (9,10). With these media, growth of cells can be obtained in up to 50% of the specimens. However, in vitro establishment of cell lines can be greatly improved by using either conditioned medium from other established cell lines (suggesting that these cells produce autostimulating factors) or by using serum-free, selective medium for SCLC (9,13).

Once established as independent cell cultures free of fibrobiast or nonmalignant cell contamination, the SCLC cells grow as either floating aggregates of cells or attached to the substrate (9–13). In many of the latter cases (more frequently seen when Waymouth's medium is used), as cell density increases, large clumps of cells detach from the surface of the culture dish and float. The general properties of the established cells of SCLC are listed in table 1. The cell lines have a relatively prolonged doubling time ranging from 36 hours to as long as 3–4 weeks, have human isoenzymes, form colonies in soft agarose at a low efficiency of 1%–5%, and form tumors in athymic nude mice with typical morphology of SCLC. The cells in culture have the typical cytologic characteristics of SCLC, intermediate cell type.

TABLE 1.—Properties of continuous cell lines of SCLC

Test	Finding
Source	Patients with SCLC
Isoenzymes	Human
Morphology in culture	Floating/adherent
Cytogenetics	Specific 3p abnormality
DDC	Present
Neurosecretory granules	Present
Peptide hormone secretion	Present
Colony-forming efficiency	1%–5% in soft agarose
Nude mouse tumorigenicity	Yes

The cell lines reveal many of the properties of APUD cells including the presence of neurosecretory granules and formaldehyde-induced fluorescence (9,10). In some cell lines, acetylcholinesterase has been demonstrated histochemically, and staining of dissociated cells of some lines for demonstration of argyrophilia has shown the presence of silver stains in some, but not all, cell lines (11).

The key APUD enzyme, L-dopa decarboxylase (E.C. 41.1.28) (DDC) has been demonstrated in both clinical specimens and cell lines of SCLC (9,14). This enzyme catalyzes the conversion of precursor amino acids (tryptophan, 5-hydroxytryptophan, and L-dopa) to their corresponding amines (tryptamine, serotonin, and dopamine). In a survey of fresh and autopsy specimens of a variety of lung cancers, although considerable overlap between SCLC tumors and non-SCLC tumors was observed, the median value for SCLC tumors was much higher than that observed in non-SCLC tumors (14,15). In contrast, among cell lines of lung cancer, the expression of DDC in SCLC lines is almost 300-fold greater than that observed in a variety of other lung cancer cell lines (14). Thus, DDC clearly differentiates SCLC cell lines from non-SCLC cell lines, at least in vitro.

IN VITRO AND IN VIVO BIOLOGIC PRODUCTS

While many neoplasms are associated with paraneoplastic syndromes, none are seen more often or have more diverse manifestations than those associated with SCLC. Ectopic production of many hormones, including adrenocorticotropic hormone (ACTH), antidiuretic hormone, calcitonin, and melanocyte-stimulating hormone, has been observed in vivo in patients with SCLC (16–24). While biochemical evidence for excessive production of these hormones may be present in up to 25% of all patients, the true incidence of clinical syndromes related to these hormones is $< 10\%$ (25).

Hormone production has also been demonstrated in continuous cell lines of SCLC (26). A large variety of hormones have been identified, including calcitonin, ACTH, parathormone, glucagon, estradiol, luteinizing hormone, somatostatin, and human chorionic gonadotropin. Although the majority of cell lines synthesized one or more of these hormones, no single hormone was produced by all cell lines (26). Clonal analysis of cell lines revealed that a single cell of SCLC can synthesize more than one hormone (11). The ability of SCLC to synthesize and secrete many hormones provides a means for evaluating factors responsible for hormone production and secretion and, potentially, mechanisms for regulating cell growth.

Because SCLC is associated with the production of many hormones, investigators have evaluated serum levels of these hormones as markers for disease extent and response to therapy in patients with SCLC. In several studies, hormones and other biologic products including ACTH (21,22), CEA (19), calcitonin (18,21), and neurophysins (20) were measured in a large number of patients at diagnosis and sequentially following the start of therapy. Serum levels were correlated with disease extent and the observed clinical response. In some studies (18–20,23), the presence of increased levels of these markers showed a good correlation with disease extent

(ie, increased levels were more frequent in patients with extensive-stage disease than in patients with limited-stage disease), while in others, no such correlation was observed (17,21,22). In addition, a close correlation between tumor response to cytotoxic therapy and serum levels of these markers was observed in those patients with an increased level at diagnosis. However, none of these markers are sufficiently specific or sensitive to achieve widespread use in the management of SCLC patients, and they rarely provide information necessary to the treatment of these patients that is not readily obtainable from physical examination or routine staging procedures. More recently, three other "markers" have been evaluated in cell lines of SCLC (27–29). Unlike most other hormones, these products—bombesin, neuron-specific enolase (NSE), and the BB isoenzyme of creatine kinase (CK-BB)—were found in most of the SCLC cell cultures and were either absent or present in very small amounts in non-SCLC lung cancer cell lines (table 2). In cultures, all three markers can clearly distinguish cell lines of SCLC origin from those of non-SCLC origin. In addition, all three substances are actively secreted by the SCLC cells, suggesting that these factors may be useful as markers in patients with SCLC. Preliminary data for both CK-BB (30) and NSE (31) serum levels in patients with SCLC demonstrate an excellent correlation with tumor burden and response to therapy.

CYTOGENETIC AND DNA CONTENT ANALYSIS

Cytogenetic analysis of solid tumors, including SCLC, has been hampered by the difficulty in obtaining sufficient metaphases in fresh specimens for study. In contrast, using flow cytometric DNA analysis, it is possible to obtain information on G_1 cell modal DNA content (ploidy) and the percentage of cells in the various stages of the cell cycle. Two recent studies have reported on the DNA content of SCLC tumor cells in clinical specimens of SCLC (32,33). In approximately 74% of the patients, an aneuploid population of tumor cells that had DNA contents ranging from 1.1 to 2.3 times diploid could be identified. In approximately 20% of the specimens with aneuploidy, more than a single aneuploid clone of cells could be identified, clearly demonstrating that, for at least some patients, SCLC is not monoclonal and that populations of tumor cells with considerable heterogeneity may exist in these patients (32).

DNA content analysis of clinical specimens may also provide a useful in vitro marker for tumor cell growth. Conflicting evidence exists for the stability of DNA content of cell lines of SCLC in culture. In a study of sequential analysis of seven tumors in vitro (34), although the DNA content of the cell lines was initially identical to that of the tumor cells in the original clinical specimen, changes in DNA content were observed in subsequent passages, with some clones disappearing and new ones emerging. In contrast, in a study of clinical specimens cloned in soft agarose and of 40 clinical specimens cultured in liquid medium for 6+ months, no significant changes in the DNA content of the tumor cells were observed, either in agarose clones or in liquid culture (35). These data suggest that frequent measurement of the DNA content of cell lines may be useful for monitoring tumor cell growth and the emergence of new clonal lines within a given tumor cell population.

Recently, cytogenetic analysis of both cell lines and fresh clinical specimens of SCLC has been reported (36,37). In one study using established SCLC cell lines, all the metaphases of the SCLC specimens had a deletion of all or a portion of the short arm of chromosome 3 (37). Shortest region of overlap analysis showed the deletion to be 3p[14–23], often expressed as an interstitial deletion. This deletion was found in cell lines established from patients prior to the start of chemotherapy, from those who had relapsed from chemotherapy, from male and female patients, and from a variety of metastatic sites. More recently, using a short-term culture (2 days) with selective growth medium (HITES) for SCLC, this deletion was identified in fresh clinical specimens of SCLC (38). This chromosome 3 abnormality was not identified in autologous diploid peripheral blood or bone marrow cells of patients whose aneuploid tumor cells demonstrated this abnormality.

TABLE 2.—Expression of bombesin, NSE, CK-BB, and DDC in cultures of SCLC and non-SCLC

	SCLC		Non-SCLC		
Marker*	No. of lines	Mean (range)	No. of lines	Mean (range)	Ref No.
Bombesin	17	1.4 (1.02–12.7)	8	< 0.01†	28
NSE	21	997 (100–9000)	9	50 (11–137)	29
CK-BB	22	7487 (2065–17,444)	12	103 (0–443)	27
DDC	22	280 (1–1415)	12	2.3 (0–42)	9,14

*Bombesin, pmol/mg of soluble cellular protein; NSE, ng/mg of protein; CK-BB, mg/mg of protein; and DDC, units/mg of protein.

†Not detectable.

Thus, this 3p abnormality is an acquired somatic cell defect that is associated with the continual replication of SCLC tumor cells. Because this defect was not observed in normal autologous blood cells or in cell lines of non-SCLC lung cancer, the defect appears to be specific for SCLC. Whether the 3p abnormality is causally related to the malignant behavior of SCLC or only associated remains to be determined.

In addition to this 3p deletion, abnormalities in chromosome number have been found in nearly every SCLC specimen, with considerable heterogeneity of chromosome numbers between SCLC lines, including hypodiploid, near diploid, hyperdiploid, tetraploid, and bimodal (37). Every chromosome except No. 18 was involved in structural aberrations, with the most frequent structural changes involving chromosomes 1, 2, 9, 10, and 14. Whether certain structural abnormalities are associated with different clinical or biologic behavior remains to be determined.

IN VITRO CLONOGENIC ASSAY

For patients with SCLC, the decision of which drug treatment to use in a particular patient is made on the basis of the results of previous clinical trials in similar patients with the same histologic type of cancer. While the majority of patients demonstrate a response to combination chemotherapy, the degree and duration of response vary. For these reasons, it is felt that there is a need to establish in vitro assays to identify cancer chemotherapeutic agents for individual patients. These assays would enable the oncologist to choose the appropriate chemotherapy for a patient's tumor in the way antibiotics are now chosen to treat infections.

In 1977, Hamburger and Salmon (39,40) reported on their success in culturing progenitor cells of human tumors. Using a two-layer, semisolid system, they successfully cultured colonies from the majority of patients with multiple myeloma and a variety of other monoclonal gammopathies. Morphologic, histochemical, and functional studies confirmed the B-cell origin of these colonies. Since that time, a number of investigators have reported on the successful in vitro cloning of a wide variety of human tumors including ovarian carcinoma, lung cancer, bladder cancer, melanoma, neuroblastoma, breast cancer, colon cancer, and a small number of lymphocytic lymphomas (41–49). Hamburger et al (42), Salmon et al (50), and Alberts et al (51), using this clonogenic assay, were able to quantitate the differential sensitivity of human tumors (ovarian carcinoma and multiple myeloma) to anticancer agents. Similar in vitro drug sensitivity data have been shown by Von Hoff et al (53) for a variety of human tumors. While the data from these studies have been encouraging, the overall accuracy for a positive prediction was approximately 60%, while the accuracy for a negative prediction was 94% (53). Thus, at present it is not certain that the in vitro clonogenic assay will predict the drug to which the patient will respond, but it may be of value in excluding drugs that are not useful.

Over the past 2 years, in vitro culture assay has been developed to assay for clonogenic cells in clinical specimens obtained from patients with SCLC (45,54,55). For these studies, specimens obtained from a variety of metastatic sites were processed for histologic and cytologic examination and for testing in the clonogenic assay. The detailed methods used for preparing and culturing SCLC specimens in 0.3% agarose over a base layer and the analysis of cultured colonies are reported elsewhere (45).

There were 250 specimens obtained from both untreated and previously treated patients. Successful in vitro colony formation of SCLC tumor cells was observed in 64 of 74 specimens (86%) containing histocytopathologic evidence of SCLC. No tumor cell colony formation was observed in specimens pathologically negative for SCLC, although typical hematopoietic stem cell colony formation was observed in a small number of bone marrow specimens. Colony formation was equally good for specimens obtained from either untreated or relapsed patients and from specimens obtained from different metastatic sites.

The origin of the colonies was confirmed by cytologic examination, electron microscopy examination, and DNA content analysis of pooled colonies (45,54,55). The reproductive or stem cell nature of agarose colonies was evaluated by intracranial inoculation of pooled colonies from ten specimens into athymic nude mice and by subculture of individual colonies from agarose into liquid medium (54). While only a few individually picked colonies (20 of 210) could be successfully established as continuous cell lines of SCLC, pooled colonies (two to 12 per mouse) from eight of ten specimens formed typical SCLC tumors in nude mice after latent periods ranging from 9 to 18 weeks, confirming the stem cell nature of these colonies.

In spite of the ability to successfully clone clinical SCLC specimens from the majority of patients, successful in vitro drug sensitivity studies were obtained in only 17 specimens (23% of all tumor-positive specimens), because of the low cloning efficiency (0.003%–0.35%) and because most clinical specimens contain only a small number of tumor cells. In vitro drug sensitivity studies using drugs with known clinical activity in SCLC were performed as previously described (50). The mean number of drugs tested in each specimen was 4.5. In the assay, the predictive accuracy for a negative prediction (in vivo resistance) was excellent (100%), while the accuracy for a positive prediction (in vivo sensitivity) was only 75%.

Because of the poor colony formation of clinical specimens, we have recently evaluated the in vitro sensi-

tivity of established cell lines to the same chemotherapeutic agents tested against fresh specimens (58). The results of this study demonstrated that these cell lines could be used to predict in vivo sensitivity. The cell lines accurately predicted in vivo resistance in 100% of the cases and in vivo sensitivity in 92% of the cases compared to the observed clinical response in patients from whom the tumor was obtained. These data suggest that cell lines of SCLC may be useful for predicting in vivo response to chemotherapy. In addition, these cell lines may provide an in vitro model for phase I–II evaluation of drugs with potential activity in SCLC (59). Preliminary studies have also demonstrated that these cell lines are an excellent in vitro model for demonstrating mechanisms of drug resistance and sensitivity in human tumors (60,61).

SELECTIVE SERUM-FREE GROWTH IN DEFINED MEDIUM

Culture media for mammalian cells usually require the presence of serum. However, the use of serum in the growth of human tumors is hampered because the serum frequently supports the growth of the nonmalignant stromal cells, which are always present in the clinical specimens, and frequently these stromal cells overgrow the neoplastic cells. In addition, serum may contain growth-inhibitory as well as stimulating factors. Other disadvantages of serum include its present high cost and the possibility of mycoplasma infection caused by improperly assayed batches of serum.

Following the work of others (62–64), we studied the replication of established cell lines of SCLC in serum-free medium supplemented with a variety of growth factors and hormones (65). After a large number of growth factors and hormones were evaluated, the combination of RPMI-1640 medium, supplemented with hydrocortisone, insulin, transferrin, 17-β-estradiol, and selenium (HITES medium), was selected. Using this combination, established cell cultures of SCLC continued to replicate in HITES medium when transferred from serum-supplemented medium (SSM), with a population doubling time approximating that in SSM. The cell lines in the HITES medium retained all the typical morphologic and biochemical characteristics of SCLC.

To further test the selective and supportive nature of the HITES medium, we evaluated the usefulness of this medium for the growth of fresh clinical specimens of SCLC and compared it with the growth of the same specimens in SSM (66). While less than one-half (44%) of 40 SCLC-positive specimens demonstrated growth in SSM, 75% (30 of 40) of the same specimens plated in HITES medium demonstrated tumor cell proliferation. In contrast to SSM, where growth of both neoplastic cells and nonneoplastic stromal cells was frequently observed, only growth of the SCLC tumor cells was observed in the HITES medium. Death and lysis of stromal cells was usually observed 7–10 days after plating in HITES medium. The tumor cells cultured in the HITES medium demonstrated all the biologic properties of SCLC cell lines cultured in SSM, including nude mouse tumorigenicity, SCLC morphology, and the expression of APUD properties (66).

The selectivity of the HITES medium for SCLC cells was shown by the failure of normal cells from 75 bone marrow specimens and of most other tumor specimens to grow in the medium; only one of 20 human tumors, including eight of nine non-SCLC specimens, demonstrated growth in HITES medium.

These data clearly demonstrate that the HITES medium is highly selective for the in vitro growth of clinical specimens of SCLC. It provides a means for the rapid augmentation of the tumor cells in clinical specimens in which the number of cells is insufficient for in vitro drug sensitivity studies. In addition, the HITES medium provides a new way for establishing new cell lines of SCLC; because of its highly selective nature and because early and rapid proliferation of tumor cells occurs in this medium, it also provides a means for performing cytogenetic analysis on clinical specimens of SCLC (38). The use of the HITES medium, because of its defined properties, will permit the easy evaluation of a large number of growth factors in SCLC and allow the purification, identification, and regulation of peptide synthesis and other SCLC products.

MONOCLONAL ANTIBODIES WITH SPECIFICITY FOR HUMAN LUNG CANCER

Using the somatic cell hybridization techniques reported by Kohler and Milstein (67) for preparing monoclonal antibodies, many investigators have generated monoclonal antibodies with specificity for a variety of human tumors, including melanoma, colon cancer, breast cancer, leukemias, neuroblastomas, lung tumors, and gliomas (68–76). Using the same techniques, monoclonal antibodies that react with a variety of human lung cancers, including SCLC and non-SCLC (75,76), have been developed. The detailed methodology for the generation and characterization of these lung cancer antibodies has been recently described (76). Monoclonal antibodies that react only with a large number of SCLC cell lines have been produced; others react with various types of human lung cancer such as adenocarcinoma and squamous cell carcinoma and with a small number of breast cancer and human neuroblastoma cell lines but not with human large cell or bronchioalveolar lung cancer cell lines (75).

These SCLC monoclonal antibodies, like monoclonal antibodies to other tumor cells, have many important clinical applications in the diagnosis and staging of patients with lung cancer and potentially in their treatment (76,77). These include the early detection and

histologic typing of tumor cells in pathology specimens; the use of isotope-labeled antibodies in the nuclear scanning of patients for the detection of occult metastatic disease; direct cytotoxicity to tumor cells; and by conjugation of cytotoxic agents (drugs, toxins, and isotypes) to antibodies, the specific targeting of these agents to tumor cells. If monoclonal antibodies are cytotoxic to tumor cells, they may be of value in the ex vivo "purification" of autologous bone marrow for subsequent transplantation, particularly in tumors such as SCLC in which bone marrow involvement is a common manifestation of the disease. All of these clinical applications of monoclonal antibodies have direct application to patient management and treatment.

HETEROTRANSPLANTATION OF SPECIMENS IN ATHYMIC NUDE MICE

Athymic nude mice are suitable recipients for the experimental heterotransplantation of many types of human tumors, including SCLC. Both clinical specimens and established cell lines of SCLC readily form tumors in these mice when inoculated by either the subcutaneous or intracranial route (56,78–81). In subcutaneous sites, SCLC tumors grow as expanding lesions, which are seldom invasive and rarely metastasize. The tumors grow progressively, reaching several grams in weight, and thus are suitable for in vivo kinetic studies of SCLC (80).

In contrast to the subcutaneous route of inoculation, intracranial tumors require 10–1000-fold fewer cells for successful take in mice (56,78,81). In addition, the latent period to tumor formation by this route is much shorter (several weeks) than that observed with subcutaneous inoculation. This observation has permitted the demonstration that in vitro agarose colonies of clinical specimens of SCLC are indeed stem cells, because in the majority of cases tested, a small number of colonies (2–12) form typical SCLC tumors when inoculated intracranially (54). The tumors formed in nude mice retain all the typical morphologic and biologic characteristics of established cultures and fresh specimens of SCLC. In addition, continuous cell lines of SCLC are readily established from these tumors. Thus, the athymic nude mouse serves as a useful mechanism for the establishment of new cell lines of SCLC and, in addition, can be used to characterize the nature of cell lines established from clinical specimens and to confirm the tumorigenic origin of agarose colonies from clinical specimens.

Several reports have demonstrated that human xenografts respond to cytotoxic agents in a manner similar to that observed clinically (82). A small number of studies using SCLC xenografts have shown a good correlation between the patients' clinical response to therapy and the response of the nude mouse tumors to similar agents, suggesting that these xenografts may be useful for predicting clinical responses. While these xenografts may be useful in evaluating new agents with potential activity in SCLC, two factors prevent the use of this model for selection of patient chemotherapy: (*a*) in most studies the successful take of subcutaneous tumors of clinical SCLC specimens is approximately 50%, and (*b*) the time required to establish the xenograft and subsequently test drugs usually exceeds the survival of the patient. Thus, for clinical purposes the major use of subcutaneous SCLC xenografts may be in the primary screening of new agents. Intracranial transplantation is a model for meningeal carcinomatosis and thus may be a useful tool to study the biology of this common clinical problem.

MEMBRANE PROTEIN PHENOTYPES

Baylin et al (83) have recently reported on the cell surface protein phenotype of the major forms of human lung cancer in vitro. Using iodinated cell membrane preparations, followed by isoelectric focusing in the horizontal axis, and then molecular weight size fractionation by SDS gel electrophoresis in the vertical axis, SCLC cells had a surface protein pattern distinct from the other types of lung cancer cells, including squamous carcinoma, adenocarcinoma, and large cell undifferentiated carcinoma. Of approximately 25 SCLC surface proteins identified with ^{125}I labeling, 12 ranging in molecular weight size from 30 to 70 kilodaltons distinguished SCLC from non-SCLC cells. Three of these 12 proteins appeared to be unique gene products for SCLC cells, as they were not present in metabolic labeling studies of non-SCLC cells or human fibroblasts. Sixteen proteins expressed on membranes of non-SCLC cells were not detected in SCLC cells. Thus, the two-dimensional gel electrophoresis system provides a new way of biochemically typing human lung cancers and also indicates that while the system is complex, there appears to be a defined group of proteins on the surface membranes of human lung cancer cells, which may prove valuable for better identification of individual cell populations in the spectrum of human lung cancers.

DISCUSSION

SCLC is a highly malignant tumor, and most patients ultimately die from the disease. Unlike the other forms of lung cancer in which surgical resection of tumors is the most effective form of therapy, SCLC is widely metastatic at diagnosis; therefore, chemotherapy alone or in combination with radiation therapy is the only effective therapy available. Our understanding of the biology of SCLC has been greatly increased by using in vitro laboratory models of SCLC to study the disease. The establishment and characterization of many cell

lines of this tumor have clearly identified many markers not previously associated with the tumor, including bombesin, NSE, and CK-BB, all of which have potential use in the clinical management of patients with SCLC. The cell lines synthesize and secrete many different hormones, thus permitting the study of mechanisms of hormonal production and secretion. In addition, studies of cell lines have confirmed that SCLC cells possess many of the properties associated with APUD cells, including neurosecretory granules, formaldehyde-induced fluorescence, and high levels of the key APUD enzyme L-dopa decarboxylase. The demonstration of these properties in anaplastic lung cancers would strongly suggest the SCLC origin of the tumor and, thus, would have a major impact on therapy selection for individual patients.

More recently, a specific cytogenetic abnormality involving chromosome 3 has been identified in all cell lines of SCLC lineage, in fresh specimens of SCLC, but not in normal autologous blood cells or in non-SCLC tumors. Confirmation of the specific defect by other investigators is essential; if confirmed, cytogenetic analysis of human lung cancer may become important in patient selection for chemotherapy.

The development of an in vitro clonogenic assay for human tumors may be useful in the selection of chemotherapy for individual patients. Although there are numerous reports on the successful in vitro cloning of many human tumors, including SCLC, there are still many problems with this assay.

(*a*) The poor cloning efficiency prevents successful in vitro drug testing in the majority of patient specimens.

(*b*) There are technical problems in obtaining a viable single-cell suspension for growth in the assay.

(*c*) Pharmacologic considerations including the proper selection of drug concentration and exposure times need to be optimized for each drug.

(*d*) Data from the assay are usually not available for 3–4 weeks.

(*e*) While the predictive accuracy for resistance in vivo is excellent, that for in vivo sensitivity is only 50%–60%.

These general problems also apply to SCLC. Before this assay can achieve widespread use in the management of cancer patients, these problems must be corrected. In addition, it is essential that prospective randomized trials are carried out to demonstrate whether the predictive value of the assay is better than results observed using optimum chemotherapy designed on the basis of previous clinical trials.

The use of in vitro models for SCLC has also permitted the identification of specific growth requirements for SCLC. Because these growth requirements in serum-free medium are selective for SCLC, they could be used for diagnosis, drug sensitivity studies in vitro, and the identification of factors regulating tumor growth. The development of monoclonal antibodies with specificity for SCLC also has potential benefit in the clinical management of patients with SCLC, both in diagnostic procedures and in the design of new therapeutic modalities for these patients. Their use offers an alternative approach in the management of SCLC patients.

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Role of Chemotherapy in Small Cell Lung Cancer: A Consensus Report of the International Association for the Study of Lung Cancer Workshop

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Many studies in small cell carcinoma of the lung (SCCL) have demonstrated a high response rate and a potential for cure in a subset of patients. Combination chemotherapy, the cornerstone of all treatment for SCCL, can produce up to 10% long-term, disease-free survival among all patients with SCCL and ≥ 20% survival among patients who present with limited disease. Significant improvements in survival have occurred in all stages of disease, and further investigative efforts are necessary to improve the complete remission rate, the duration of response, and the percentage of long-term, disease-free survivors. The current report, based on the workshop on SCCL held in Ireland under the auspices of the International Association for the Study of Lung Cancer, reviews the current state of the art for chemotherapy and presents potential future directions. [Cancer Treat Rep 67:37–43, 1983]

Considerable attention has been focused on small cell carcinoma of the lung (SCCL) because of its unique histology, biologic behavior, and response to a variety of treatments (1). Multiple combination chemotherapy regimens alone or combined with chest irradiation have produced clinical results that show significant palliation for nearly all patients with this disease. Many studies also show a small number of long-term survivors, suggesting the potential for cure. To define current status and future direction in SCCL, a workshop was held under the auspices of the International Association for the Study of Lung Cancer. The purpose of this paper is to present the consensus report of the chemotherapy committee relative to the current status of chemotherapy, minimal expectations of results and methods of reporting, current concepts of ongoing studies, and potential new directions.

MINIMAL ANTICIPATIONS

It has been clear for many years that SCCL is highly responsive to radiation therapy and multiple chemotherapeutic agents (2,3). A relatively large number of combination chemotherapy studies utilizing multiple combinations of the pool of active agents have been reported (1–5). These combinations have been used either alone or together with radiation therapy as part of combined-modality treatment, and they have produced a relatively narrow spectrum of results, which can be summarized as follows.

(*a*) When treatment is minimally aggressive (with little toxicity), the results are not maximal (1–3,6). Thus, more aggressive chemotherapy regimens appear to produce slightly better response rates, durations of response, and numbers of patients who achieve long-term, disease-free status (plateau) (1–5,7–11).

(*b*) Multiple drugs appear to be superior to single agents, and three drugs appear to be superior to two (4,5,12,13). Continued improvement may be seen by the addition of more agents beyond three (12), but this may depend on both the types of agents and the aggressiveness of therapy. Future prospective studies have yet to demonstrate this relationship.

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(*c*) Results of treatment according to disease status (extent) are a reflection of the staging studies that are used to determine extent of disease (14) and the restaging studies utilized to document the extent of response (*see* report by the prognostic features, staging, and evaluation committee).

(*d*) To deliver maximal therapy, it is necessary that a certain level of toxicity be achieved, with a regular and predictable fall of hematologic parameters. This results in infectious episodes during the period of granulocytopenia and in occasional toxic deaths. It has not been determined whether any given combination has a steep dose-response curve that warrants even more aggressive therapy. Thus, whether autologous marrow rescue after lethal or supralethal combination chemotherapy has any utility is yet to be demonstrated.

GOALS AND ANTICIPATED RESULTS

The eventual goal in the treatment of SCCL is to develop effective systemic therapy that will produce high response rates and a high percentage of long-term, disease-free survivors (cures). Currently, the results that can be obtained with maximally delivered combination chemotherapy (with or without additional radiotherapy) are limited by the available agents. Certain expectations, however, can be derived from what might be considered state-of-the-art chemotherapy, as defined in many studies, particularly large single-institution studies in which the staging, evaluation, and treatment are usually performed by the same individuals. Although some of the cooperative group studies have reported results with lower response rates and a lower percentage of long-term survivors, the consistent results of multiple individual studies suggest that these are clearly obtainable goals. Most combination chemotherapy regimens produce > 80% total response, ie, complete response (CR) plus partial response (PR) rates, in all stages of SCCL (1–11). Total response determination, however, is insufficient in analyzing the results of therapy. Further delineation with respect to CR is necessary to adequately assess the results of therapy. Assuming that maximal staging procedures have been utilized to differentiate limited and extensive disease, current aggressive combination chemotherapy should produce CR in > 50% of the patients with limited disease and in > 20% of the patients with extensive disease. Assuming adequate staging, the median survival should be ≥ 14 months in patients with limited disease and > 7 months in patients with extensive disease. With respect to long-term survival, approximately 15%–20% of the patients with limited disease should be expected to achieve long-term, disease-free survival (≥ 3 years) 1–5,7–9). Currently, few patients with extensive disease have such long-term, disease-free survival. If, however, staging procedures have not been maximized, the results for limited and extensive disease begin to approach one another. However, one may still look to the total population for anticipated results. This would include a 40%–50% CR rate for the entire population, a median survival of approximately 12 months, and an overall plateau of approximately 5%–10%, depending on the admixture of limited and extensive disease. These results have now been achieved in a large number of studies utilizing multiple combinations (1–5,7–12,15,16). The similarity and repeatability of these results suggest that any results less than these be considered suboptimal.

ANTICIPATED REPORTING

Sufficient studies have now been reported to make certain features of future reports highly desirable, including appropriate stratification of prognostic factors known to influence the disease course such as performance status and extent of disease (14). Level of response (CR or PR) should be clearly specified for both limited and extensive disease and, if possible, according to performance status. The restaging procedures utilized to determine level of response should also be clearly defined. Another highly desirable item for inclusion in reports of treatment would be the time to the attainment of CR. This would allow for correlation between the time to CR and such factors as duration of response and subsequent responsiveness.

An additional aspect of reporting that would be useful in subsequent analysis and for comparison between studies is the inclusion of sites of relapse, particularly among patients in CR. Such reporting would allow for standardization of patterns of relapse and local control.

Most studies also show certain common features.

(*a*) Patients with CR have significantly longer survival than patients with PR (or less than CR), and analyses and demonstration of such curves should no longer be considered absolutely necessary.

(*b*) Patients with limited disease have significantly better survival than patients with extensive disease and, similarly, such survival comparisons need no longer be graphically illustrated.

Certain aspects of graphic illustration have led to some confusion regarding long-term survival, and this has been the use of the early projected actuarial survival curves. There are published studies showing projected survival curves that are much higher than the final survival data (9,17,18). This suggests that actuarial survival curves should not be published until there is sufficient follow-up of the entire population to warrant an accurate representation of the survival curve. One approach to solve this problem would be to publish

only the final survival curve. Another would be to publish an actuarial survival curve after the last patient entered has been followed for at least the median duration of survival.

TOXICITY

With the understanding that maximally delivered combination chemotherapy (with or without irradiation) is currently necessary to achieve state-of-the-art results, it can be anticipated that some serious complications will ensue (19). In most studies these include drug-specific toxic effects (eg, renal toxicity from platinum and neurotoxicity from vincristine) in addition to myelosuppression and serious infectious episodes during periods of granulocytopenia. Most studies that have utilized aggressive combination chemotherapy have, however, reported that such serious complications occur in $\leq 25\%$ of the treated patients, that lethal toxic effects occur in $\leq 5\%$, and that the toxic effects may be a function of poor performance status. For the purposes of reporting toxicity, it would be useful if the median of the lowest and all wbc and platelet count nadirs, the duration of such myelosuppression, and the specific cause of toxic deaths could be given. Other toxic effects should be categorized according to the specific drug or treatment to which they are ascribed.

CURRENT CHEMOTHERAPY STUDIES

Since combination chemotherapy (with or without additional radiotherapy) can produce significant palliation and a small percentage of long-term, disease-free survivors, a number of studies are currently under way to determine if other treatment approaches can improve the currently achievable results. These include:

(*a*) the prospective evaluation of the use of adjuvant radiotherapy for local disease (4,5,20),
(*b*) the evaluation of alternating non-cross-resistant combination chemotherapy,
(*c*) the evaluation of dose-schedule dependency for some of the active agents,
(*d*) the determination of dose-response relationships using intensive treatment and maximal supportive care,
(*e*) the use of anticoagulants, and
(*f*) the role of total parenteral nutrition as part of a program of combination chemotherapy.

Adjuvant Radiotherapy

Since several studies have demonstrated that radiotherapy offers little to total disease control or survival in patients with extensive disease, several prospectively randomized studies are currently under way to test the role of radiotherapy as an adjuvant to chemotherapy in limited-disease SCCL. The details of these studies and the logic for their design and evaluation will be presented by the report of the radiotherapy committee.

Alternating Non-Cross-Resistant Chemotherapy

If one combination chemotherapy regimen were shown to be effective in patients who had failed another effective combination chemotherapy regimen, then it is conceivable that the two regimens might be alternated to augment response rate, duration of disease control, or survival. At least nine "alternating, non-cross-resistant" combination trials have been instituted or completed (7,21–28). The difficulty with the interpretation from both the preliminary and final results of many of these studies lies in the definition of alternating therapy and in the identification that the two combinations are effective and non-cross-resistant. First, both combinations should have comparable response rates as first-line therapy. Second, each combination should produce high-level responses (ie, CR) in patients refractory to the other treatment. Furthermore, alternating combinations could be utilized early, with the second combination beginning immediately after the first combination (in a sequence such as ABAB), or late (delayed), with the first combination used for induction of maximal response and the second used in an attempt to augment the response achieved with the first-line combination (in a sequence such as AAABAB or other permutations). Thus, it is not surprising that the various alternating chemotherapy studies have failed to show uniform results. It is entirely conceivable that if the first combination were less effective as first-line therapy than the second, then such a study would show response enhancement with the alternating treatment as opposed to a nonalternating treatment. On the other hand, if the first treatment modality were the more effective, then it would be conceivable that the second therapy would offer little improvement in response compared to a nonalternating sequence. Regardless of the theoretic discrepancies among the results of the various studies, it is apparent for all of the alternating non-cross-resistant studies, regardless of whether positive or negative, that the best arm of all of these studies produces approximately the current state of the art as addressed above. Thus, although all of the ramifications of alternating non-cross-resistant combinations have not been completely settled, it appears that another approach will probably be necessary to markedly change the results.

Dose-Schedule Considerations

The determination of maximum dose for the various active agents follows from the concept of utilizing max-

imal therapy. This has been addressed for some agents and is in progress for others. Schedule dependency, however, has been evaluated less commonly. For cyclophosphamide, there appears to be an increasing response with increased dose, but the dose-response curve appears to plateau at ≤ 1.5 g/m^2 (21,29–34). Doxorubicin appears to have a maximum dose-response relationship in the range of 45–60 mg/m^2 per course (30,31,34). Similarly, high doses of methotrexate with folinic acid "rescue" have not yet proved to be more efficacious than conventional dose methotrexate (without rescue) (35). On the other hand, etoposide appears to have both a dose and schedule dependency in animal systems and possibly in man (36,37). Thus, at least two studies are currently testing the maximal utilization of etoposide by dose and schedule (38,39).

Another approach to evaluate dose-response relationships is to utilize ablative high-dose chemotherapy and autologous bone marrow "rescue." Bone marrow is harvested during remission and assayed for tumor content by either DNA flow cytometry, growth in soft agar (40,41), or storage in liquid nitrogen for a finite period of time to assure that the patient has not had a bone marrow relapse. Subsequently, the patient is given very high (lethal or supralethal) doses of chemotherapy, and the autologous marrow is reinfused, allowing for a marrow recovery. In the United Kingdom and elsewhere, several centers are also studying the use of autologous marrow rescue following 6–24 hours of short-term storage in liquid phase. Results from several such studies have clearly shown that the technology is feasible, but they have yet to demonstrate that this technique produces end results superior to those obtained by maximal administration of chemotherapy without the use of autologous marrow reinfusion (42,43).

Total Parenteral Nutrition

Since many patients initially have weight loss, which is an adverse prognostic factor, another approach has been to determine if an increase in total protein-calorie intake can augment chemotherapeutic responsiveness and toxicity. In a multi-institutional trial, patients receive the same combination chemotherapy and are randomized to receive total parenteral nutrition by iv hyperalimentation. The preliminary reports from these studies suggest that results, again, are not superior to those obtained with currently available chemotherapy, and it is unclear what, if any, role hyperalimentation plays in the overall management of this disease (44). Further analysis of the current studies, however, is necessary to exactly define the utility of this technology.

Anticoagulants

Another interesting observation has been related to the use of anticoagulants together with combination chemotherapy. The rationale for this approach is the possible perturbation of metastases formation within microscopic clots. A recent randomized study has suggested that this combination is more effective than chemotherapy alone (45). The problem remains, however, that there were accrual difficulties with the study, and the control arm achieved lesser results than can be obtained with maximally delivered chemotherapy. This has, however, stimulated further studies in this area, including a prospectively randomized study by the Cancer and Leukemia Group B.

DURATION OF THERAPY

Since many combination chemotherapies can produce similar high response rates, similar durations of survival, and similar numbers of long-term survivors, one must ask how long chemotherapy should continue. Some studies have suggested that all patients who achieve CR will do so within the first 6 weeks (two courses) (29), whereas other studies have shown that patients may achieve CR with as many as six courses of combination chemotherapy (7,21,33). Some recent studies have suggested that an intensive induction lasting for six courses, without subsequent maintenance, may provide reasonable response and survival results (46,47). Thus, it appears that a minimum of six courses of combination chemotherapy should be administered.

Questions remain regarding whether the duration of treatment should be the same for both limited and extensive disease and whether there should be a distinction between those who achieve CR and PR. Firm guidelines for continuation of therapy are not easily determined, and various studies have ended chemotherapy at 6–30 months. There are few relapses among patients with limited disease who remain disease-free for > 24 months. However, there appears to be a relatively constant rate of relapse from time of CR to 24 months, suggesting that maintenance therapy may have a limited role in the continuation of response. Such questions lend themselves easily to study, and a prospectively randomized evaluation of the timing of treatment discontinuation would be worthwhile to minimize the toxicity and inconvenience associated with aggressive combination chemotherapy. Such a study would seek the minimal therapy needed to achieve current results. However, to improve results, it is likely that even more aggressive treatment may be needed.

NEW APPROACHES

Since preliminary results from alternating combination chemotherapy and dose-response studies have not shown major improvements in obtainable results, new approaches are clearly needed to achieve a change in the overall disease-free survival.

Late Intensification

One approach for the chemotherapeutic treatment may be derived from the mathematic model of Norton and Simon (48), who suggest that therapy subsequent to induction may need to be intensified. Thus, this is one rationale for a program of late intensification chemotherapy. This concept, in part, is substantiated by the constant relapse rate that occurs after the induction of CR. Thus, a late intensification program utilizing high-dose chemotherapy or high-dose chemotherapy plus irradiation may be useful to attempt to consolidate the remission state and prolong both remission and duration of disease-free survival. One of the difficulties in this particular process will be the selection of appropriate controls, since those patients who have been chosen for late intensification of therapy have already had a period of disease-free survival and may thus be preselected as a more favorable group. Thus, such studies must be carefully planned to avoid a biased selection of the group receiving late intensification, which may take the form of either chemotherapy or irradiation. A preliminary report (49) from the use of late chest irradiation for patients who achieved CR suggests that this may improve local control, but further follow-up is necessary to determine the impact on duration of control and survival as well as patterns of relapse.

Chemotherapy Plus Surgery

Another approach in the treatment of small cell carcinoma, particularly for those patients with limited disease, has been to re-examine the role of surgery as part of the overall treatment approach. For isolated intrathoracic lesions, patients undergo careful evaluation, total resection, and subsequent chemotherapy. Such an approach can be justified by the results of the "coin lesion" study (50), which showed a high fatality rate in patients with SCCL who presented with isolated intrathoracic lesions. Patients who present with greater bulk of disease including ipsilateral mediastinal disease in the chest receive preoperative chemotherapy and then undergo total extirpation of visible disease at thoracotomy (51,52). A number of useful observations are likely to come from this approach of examining gross and microscopic pathology from postchemotherapy specimens, with evaluation of abscess and fibrosis formation, evidence of residual disease within the fibrosis, and potential changes in histology on both light and electron microscopy. Such a surgical approach may also permit the correlation of biologic behavior of the tumor by study in vitro of specimens obtained before and after chemotherapy exposure in vivo.

Several collaborators in this study are utilizing the TNM staging system before chemotherapy as well as prior to and after surgery, to allow for closer correlation of end results with postchemotherapy stage and treatment. Because only preliminary results are available, it is too early for any conclusions regarding the relative efficacy of this approach, and further results are necessary to determine its value.

NEW DRUG DEVELOPMENT

One of the major promises for future treatment of SCCL remains the development of active new agents. New drug testing is an important and integral part of SCCL treatment programs. Recently, there have been a large number of negative phase II trials of new drugs in SCCL. This may be a result of the inactivity of these drugs in SCCL, or it may occur because the activity of all drugs following first-line intensive induction chemotherapy is much diminished. In other words, it is conceivable that aggressive first-line chemotherapy may alter the response of SCCL so that it becomes more refractory to subsequent therapy with any drug. There are several good illustrations of this phenomenon with drugs such as etoposide and vindesine; when used in patients who had been minimally treated or untreated, these drugs produced reasonable response rates (36,37,53,54), whereas in a series of patients with SCCL who had been aggressively treated, the response rate for etoposide was $< 20\%$ (B. Issell, personal communication) and was negative for vindesine (55,56). This difference in response depending on prior treatment poses significant difficulties for new drug development and raises serious questions about the approach that should be utilized to test new drugs. In view of the lesser responses seen in previously treated patients, it may be necessary to test new drugs in a larger population of patients during phase II trials and to determine a $< 10\%$ activity level with 95% confidence rather than the 20% activity level that is usually sought in such trials. An alternative might be to use previously untreated patients. The standard approach, ie, utilizing new drugs after conventional drugs have failed, could leave considerable doubt regarding possible activity of reportedly inactive drugs. On the other hand, since established combination chemotherapy can provide significant palliation for many patients, it is difficult to deny any patient the opportunity for response in order to test new drugs. These are difficult and serious questions for which there are no easy answers.

Some of the answers may come from the laboratory models that have been developed for in vitro growth of SCCL, eg, the clonogenic assay and similar in vitro cell lines. Should a new drug prove to be promising under these circumstances, it could be tested in patients who fail primary therapy or possibly in a subset of previously untreated patients (*see* below). In treating patients who have recurrence or are resistant to primary therapy, a distinction should be made between those who have recurrence after some response and are then

refractory to primary therapy and those who had primary resistance to initial therapy, since this probably signifies different biologic behavior.

Another approach to new drug testing might be to add a new agent to an established combination as part of a front-line combination. This method was used by Eagen et al (57) to test the activity of platinum as part of polychemotherapy for SCCL. This approach, however, requires a relatively large number of patients committed to the identification of the activity of a drug and would require many more patients to show inactivity than the usual phase II criteria for drug evaluation.

Still another approach might be to utilize a subgroup of patients with relatively good performance status and extensive disease who are likely, by virtue of having no visceral crisis, to survive a short period of new drug testing and then receive conventional therapy. Such an approach can be justified since (*a*) there are virtually no long-term survivors among patients with extensive disease and (*b*) there would be laboratory indications to suggest activity for this drug, providing for rapid drug screening in the most clinically efficient manner.

Regardless of the approach for testing new drugs, it is clear that patients must have as good a performance status and must be as early in treatment as possible for evaluation of new drugs. Since SCCL must be considered a systemic disease process, it is likely that the identification of new active treatments (chemotherapeutic agents) is one of the cornerstones that will enable us to improve the overall treatment results of this dread disease.

New Modalities

In addition to drug development, it is necessary to investigate all potential avenues and new modalities of treatment, such as hyperthermia, systemic radiotherapy, the role of continued smoking and its biologic consequences, or the identification of factors that may regulate the growth and maturation of SCCL both in vitro and in vivo. These are important future developments, which await technologic applications. Hopefully, the systematic evaluation of all treatment modalities alone and in combination, the application of new drugs, and the testing of all permutations of dose-response relationships, as well as the identification of more effective systemic therapy, will result in an improved percentage of long-term, disease-free survivors. It appears that SCCL, now shown to be a potentially curable disease, can be converted to a much more readily curable disease by appropriate measures and systematic investigation of therapeutic alternatives.

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Model Studies in Adjuvant Chemotherapy[1,2,3]

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We have focused on three aspects of adjuvant chemotherapy applied to mice with one of the metastasizing tumors: Lewis lung carcinoma (LL) or mammary carcinoma 2661 (M2661). The first aspect concerned the timing of adjuvant chemotherapy. To investigate this, tumor-bearing mice were treated postoperatively with cyclophosphamide using a standard regimen. In M2661, adjuvant therapy was marginally effective in contrast to the clearly significant results obtained in LL. Any delay in the initiation of adjuvant therapy decreased the efficacy of the treatment. The effect of administering chemotherapy before surgery was also studied; normally, marginally effective adjuvant therapy was found to become effective when started preoperatively in M2661. In LL, effective adjuvant therapy was found to become less effective when started preoperatively. The second aspect considered was the comparability between the increase in relapse-free survival time and the increase in cure rate as alternate goals of adjuvant therapy. To study this, mice with small, medium, or large residual tumor loads were subjected to surgery and subsequently treated with cyclophosphamide. While the effect of adjuvant therapy on the cure rate increased proportionally with decreasing tumor load, the increase in lifespan in nonsurvivors was not related to tumor load. The final aspect of study was the selection procedure for drugs to be applied in adjuvant treatment in our models. Taking the volume response of large tumors as being predictive for the successful use of the same agent in adjuvant therapy, we obtained both false-negative and false-positive results in our tumor lines. [Cancer Treat Rep 67:45–50, 1983]

The selection of drugs to be used in adjuvant chemotherapy regimens is generally made on the basis of their activity in advanced disease. For example, because 5-FU is the most effective drug in advanced colorectal tumors, it has also been used in almost all adjuvant protocols for this type of disease. Although this practice of extrapolation of results obtained in the late stage of the disease to the early stages seems sensible, the criterion of selection may exclude active drugs for adjuvant therapy.

Another basic problem the chemotherapist has to consider when applying adjuvant chemotherapy is which group of patients should be selected for the adjuvant treatment. Mammary carcinoma patients with one axillary lymph node metastasis (N1+) have a low risk of developing recurrent disease. It may be unwarranted to apply intensive adjuvant chemotherapy in this N1+ population, since this will be overtreatment in > 50% of such patients. In contrast, patients with four positive lymph nodes (N4+) have a high risk of relapse, and a treatment aimed at sterilizing micrometastases seems fully justified. The high-risk patient is always selected for adjuvant trials, not only in mammary carcinoma but also in colorectal tumors, ovarian tumors, and melanomas.

These commonly applied procedures were investigated in tumor-bearing mice treated postoperatively with cyclophosphamide (cyclo).

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MATERIALS AND METHODS

Tumor lines.—Mammary carcinoma 2661 (M2661) transplanted in CBA/Rij mice is a nonimmunogenic tumor with a tumor volume doubling time of 2.5 days. After inoculation of 10^5 tumor cells into the foot pad, lymph node and pulmonary metastases develop (1). Lewis lung carcinoma (LL) maintained and transplanted in C57BL/Ka mice is a weakly immunogenic tumor (2,3). The tumor volume doubling time is 2 days. After inoculation of 10^5 cells into the foot pad, this tumor metastasizes to the lung and on rare occasions to regional lymph nodes.

Treatment.—Removal of the primary foot pad tumors was accomplished by amputation of the tumor-bearing right hind leg at the knee joint while the animals were anesthetized with ether. The amputation was performed in the third week after M2661 or LL cell inoculation. To avoid increased spread of tumor cells, the level of amputation was chosen in such a way that neither the foot pad tumor nor the area of popliteal lymph nodes was damaged. Non-tumor-bearing mice were excluded from the experiments (< 1% of the inoculated population). For the assessment of the effect of adjuvant chemotherapy, cyclo (provided by Asta-Werke) was injected ip 3 days (d+3) after amputation. In the M2661 studies, the highest nontoxic dose of cyclo (200 mg/kg ip; LD10, 250 mg/kg ip in tumor-bearing mice) was applied, since lower doses were ineffective in the adjuvant experiments. Since the LL was very sensitive to cyclo therapy, we applied a moderate dose of 100 mg/kg ip in all of the LL experiments. To establish a correlation between the efficacy of cyclo treatment and the extent of delay in starting this therapy, the drug was administered 1 day before amputation (d–1) or at various time intervals after amputation (eg, d+7). From primary tumor characteristics such as tumor volume (T classification), the risk of dying from metastatic disease can be predicted. As a rule, the more advanced locally (T3), the larger the residual metastatic tumor load after amputation of the tumor-bearing leg. To study the residual tumor load dependency of adjuvant therapy, cyclo was administered in carefully staged groups of mice with low, medium, and high residual loads of metastatic disease. The aim was to establish a correlation between primary tumor volume at the time of surgical intervention (T classification) and the increase in lifespan (ILS) after adjuvant therapy. The effect of cyclo treatment on the primary tumor was investigated by treating foot pad-inoculated tumor-bearing mice. The drug was administered either very early in the course of the disease, on Day 3 after tumor cell inoculation, or at a later time when the tumor was palpable, eg, on Day 15 after inoculation (primary tumor size dependency experiments).

Endpoints and statistical evaluation.—The endpoint for cyclo-treated primary tumors was growth delay and local cure rate. Tumor volume was expressed as the product of the three measured diameters of the foot pad tumors (a × b × c). The growth delay in days is defined as the displacement in time between the growth curve of the tumors in the control group and that of the tumors recurring after treatment. The displacement between the growth curves is chosen at a point (usually close to the original tumor volume) where the growth curves for treated and untreated control animals are again parallel. If no original tumor volume is known (eg, for tumor inocula exposed to cyclo treatment before a tumor is palpable), the delay in comparison with the untreated control group to reach an arbitrary volume of between 100 and 300 mm^3 is estimated. In the M2661 studies, death was caused by the outgrowth of nodal and/or lung metastases; in the LL experiments, mice died of lung metastases. Death from metastatic disease was observed to occur within 3 months at the most and, consequently, 100-day survivors were assumed to be cured. The survival time of mice after postoperative chemotherapy correlates with the effect of treatment on the residual tumor. The log-rank test was used for the statistical evaluation (4). Two endpoints were considered in the adjuvant therapy experiments: the ILS after amputation and the increase in cure rate. The % ILS (death delay) was determined by using the median survival time (MST) after amputation of the nonsurvivors in the equation:

$$\%\ \text{ILS} = \frac{\text{MST of treated animals} - \text{MST of surgical controls}}{\text{MST of surgical controls}} \times 100\%.$$

The increase in percent cure rate is the difference between the percent cure rate in treated mice and in surgical control mice. Under the experimental conditions, the nonsurvivors died of relapses and, consequently, the reduction in relapse rate calculated from the animals at risk is a relevant endpoint of cyclo adjuvant chemotherapy. The relapse rate reduction was determined by the equation:

$$\frac{\text{relapse in surgical controls} - \text{relapse in treated animals}}{\text{relapse in surgical controls}} \times 100\%.$$

Student's t test was used for the statistical evaluation of primary tumor responses. With the cyclo dosages applied, no animals died of drug-induced toxicity.

RESULTS

Randomly selected mice with foot pad tumor volumes of between 100 and 400 mm^3 were subjected to amputation. Cyclo treatment was administered on the third postoperative day (d+3). As shown in table 1, in the M2661 studies, the ILS after cyclo adjuvant therapy (200 mg/kg) was negligible ($P = 0.4$). In the LL studies, however, the efficacy of cyclo adjuvant therapy (100

TABLE 1.—Time dependency: effect of cyclo timing in combination with amputation*

	Timing of cyclo		
	Adjuvant (d + 3)	Delayed adjuvant (d + 12)	Preoperative (d–1)
	M2661 (200 mg/kg of cyclo)		
% ILS (days)	13 (4)	53 (16)	70 (21)
% relapse rate reduction†	8	–3	5
P value‡	0.4	0.7	< 0.01
	LL (100 mg/kg of cyclo)		
% ILS (days)	57 (23)	15 (6)	37 (15)
% relapse rate reduction†	82	46	12
P value ‡	< 0.001	< 0.01	< 0.001

*Cyclo was administered before (d–1) or after (d + 3 or d + 12) amputation performed on Day 21 following tumor cell inoculation. Mice died of metastases. The MST of nonsurvivors in control animals was 30 days for M2661 and 40 days for LL.

†Differences (*P* values) between survival curves for surgical control and cyclo-treated animals were calculated by use of the log-rank test. Cumulative data of at least 3 experiments/data point are presented, each involving a minimum of 50 mice.

‡As determined by: (% relapse in surgical controls – % relapse in treated animals)/(% relapse in surgical controls).

mg/kg) was highly significant. Table 1 also demonstrates the effect of delaying the cyclo administration. A delay of 9 days before cyclo treatment in the LL group considerably decreased the efficacy of the therapy. Since the results of postoperative cyclo therapy in the M2661 group were disappointing, we investigated whether giving the drug preoperatively (d–1) would have an effect on survival. As shown in table 1, cyclo therapy was effective ($P < 0.01$) for M2661 only when the drug was applied preoperatively at a time when the metastatic load was presumably very small. In contrast, preoperative treatment in the LL group was not only less effective than early adjuvant therapy but also less effective than delayed adjuvant therapy.

Instead of administering the cyclo therapy at an earlier time and thereby artificially decreasing the residual tumor load, our next step was to perform experiments in which animals were preoperatively stratified according to their primary tumor volume. Survival curves of surgical control, non-cyclo-treated mice are presented in figure 1. Mice with small T1 foot pad tumors at the time of amputation (~ 100 mm^3) had, according to the curve, a minimal residual tumor load, since > 50% survived without any form of adjuvant therapy. In contrast, mice operated on for T3 or T4 tumors had a large residual load, since > 80% of the animals died within 50 days after amputation. Cyclo treatment given 3 days after amputation resulted in an effect which was tumor load-dependent. This effect on lifespan is the result of an increase in the survival time of nonsurvivors (death delay) and an increase in cure rate. These two effects were determined separately by calculating for the nonsurvivors the % ILS and the difference in percent cures in treated animals and surgical control mice. The reduction in relapse rate as a result of cyclo treatment was determined by the equation described earlier. The results of a standard treatment with cyclo at 3 days after amputation in mice with tumors of various T categories are presented in table 2. The average difference between the MST in surgical controls and cyclo-treated mice with tumors of all T categories was 8 days for LL and 14 days for M2661. Consequently, the average ILS was 48% in the M2661 group and 20% in the LL group. The % ILS was not significantly related to the T category. The increase in percent of mice cured is also presented. The effect of cyclo on the reduction in relapse rate was calculated for both tumor lines. In the M2661 group, the relapse rate reduction decreased with increasing T category from 16% to 0%. In the LL group, the reduction was from 66% to 11%. The data from these residual load experiments indicate that, with a standard cyclo adjuvant regimen, the decrease in relapse rate is more pronounced in animals with a low average residual tumor load. The decrease is residual tumor load-dependent. There is, however, a marked difference in the effect on the shape of the survival curve in the two tumor lines. In the M2661 group, cyclo affected the ILS more than the cure rate, and the opposite was observed in the LL group. This is shown for M2661 in figure 2.

In other experiments (table 3), we investigated the effect of cyclo on tumors of various sizes (primary tumor-size dependency experiments). Mice with foot pad tumors were treated at a stage when the tumor was not palpable (Day 2 after tumor cell inoculation) or at a later stage when the tumor had a volume of approximately 300 mm^3. When nonpalpable tumors were treated, the

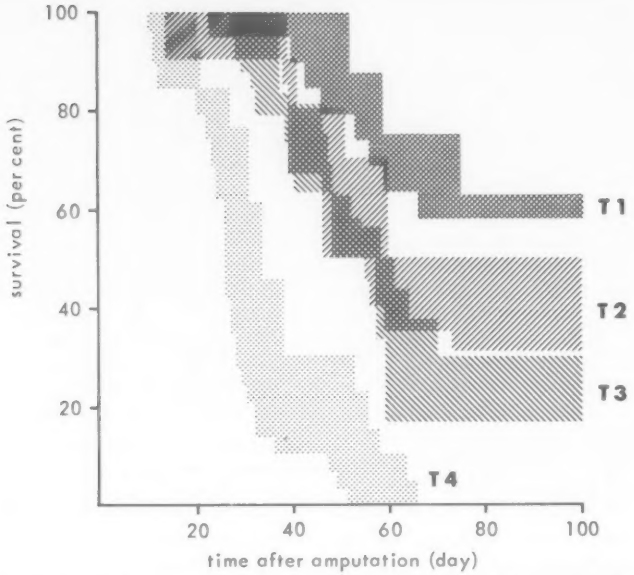

FIGURE 1.—At about 3 wks after LL cell inoculation into the foot pad, tumor-bearing mice were stratified according to the volume of their primary tumor and subjected to amputation (T1, < 100; T2, 100–200; T3, 200–300; and T4, > 300 mm^3). Thereafter, no further treatment was given. Mice died of lung metastases. Each of the 4 shaded areas represents the range of the survival curves for each of the T stratifications. For T1, the range of 2 experiments is presented; for T2, T3, and T4 tumors, the ranges of 4 experiments are presented.

M2661 responded with a mean growth delay of 26.7 days and 10% local cures. Medium-sized tumors responded less markedly to cyclo therapy: the mean growth delay was decreased to only 4.2 days. This tumor size-dependent effectiveness of cyclo was also demonstrable in the LL group. Treatment of unpalpable LL was more effective than the treatment of older or larger tumors.

When the primary tumor response (table 3) is compared with the micrometastases response as determined by the survival time of mice (table 1), there seems to be a discrepancy. The primary M2661 tumor is sensitive to cyclo therapy; the micrometastases, however, are cyclo-resistant in most of the adjuvant experiments.

Based on these observed discrepancies between pri-

TABLE 2.—Load dependency: residual tumor load-dependent effectiveness of cyclo adjuvant therapy*

T category	MST (days)		% cures			
	Surgical controls	Cyclo-treated mice	Surgical controls	Cyclo-treated mice	*P* value†	% relapse rate reduction‡
			LL			
T1 (n = 4)	61	54	62	87	0.3	66
T2 (n = 19)	47	56	35	67	< 0.02	53
T3 (n = 24)	40	48	12	29	< 0.02	20
T4 (n = 28)	32	39	3	14	< 0.04	11
			M2661			
T1 (n = 25)	37	51	8	23	< 0.005	16
T2,3 (n = 40)	27	48	2	15	< 0.0005	13
T4 (n = 10)	28	36	0	0	< 0.02	0

*Tumor-bearing mice were preoperatively stratified according to their primary tumor volume (T1, < 100; T2, 100–200; T3, 200–300; and T4, > 300 mm^3). Cyclo adjuvant therapy was administered 3 days after amputation. Mice died of metastastic relapses or were cured. n = average No. of mice/treatment group.

†*P* = significance of difference in the log-rank test.

‡As determined by: (% relapse in surgical controls – % relapse in treated mice)/(% relapse in surgical controls).

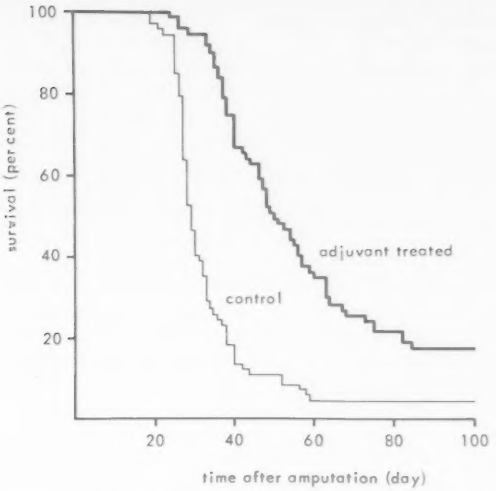

FIGURE 2.—M2661 survival curves. Mice bearing various residual tumor loads were followed until death. The M2661 localized in the foot pad was amputated at about 3 wks after tumor cell inoculation. At the time of primary tumor treatment, the volume of the tumors varied between 100 and 300 mm^3. Cyclo adjuvant therapy was applied at 3 days after amputation.

mary tumor and micrometastases responses, experiments in which very large primary tumors (~ 500 mm^3) were treated with cyclo were designed. The cyclo was given 7 days before the amputation was performed to make possible the determination of the local tumor volume response to the cyclo treatment. The results presented in table 4 indicate that neither tumor volume reduction nor growth delay could be determined in either the M2661 or the LL groups, suggesting cyclo resistance in both tumor lines. The simultaneous systemic effect of cyclo, however, was significant ($P < 0.02$ and $P < 0.0003$, respectively). These effects of cyclo on the primary tumor and on the lifespan of mice operated on for large primary tumors are in agreement with the

TABLE 3.—Size dependency: relation between tumor volume and response to cyclo*

	Volume at time of cyclo treatment	
	Not yet palpable	Palpable (300 mm^3)
M2661 (200 mg/kg of cyclo)		
Growth delay (days)	26.7	4.2
% local cures	10	0
LL (100 mg/kg of cyclo)		
Growth delay (days)	2.4	0.5
% local cures	33	0

*Cyclo was administered on Day 2 or between Days 12 and 16 after tumor cell inoculation. Cumulative data of at least 5 experiments each involving a minimum of 20 mice are presented.

TABLE 4.—Discrepancy between very large primary tumor volume response and micrometastases response*

	Response to preoperative cyclo treatment	
Endpoints	M2661	LL
Primary tumor response		
Growth delay	None	None
Micrometastases response		
ILS of nonsurvivors	32% (9 days)	55% (15 days)
Relapse rate reduction†	0% $P < 0.02$	5% $P < 0.0003$

*Selected mice (n = 50) with large primary foot pad tumors (> 500 mm^3) were treated with cyclo on Day 21 and this was followed by amputation on Day 28. Mice died of metastases or survived > 100 days. P values were calculated by use of the log-rank test. MST of nonsurvivors was calculated from day of amputation, and % relapse in surgical control animals was 100% (28 days) in the M2661 group and 96% (27 days) in the LL group.

†As determined by: (% relapse in surgical controls – % relapse in treated mice)/(relapse in surgical controls).

data shown in tables 1 and 3. Therefore, our tumor cell lines are sensitive or resistant to cyclo therapy, depending on the type of experiment or endpoint used.

DISCUSSION

In the adjuvant experiments presented in table 1, the mice were not stratified according to their primary tumor volume; consequently, mice with widely different residual loads were treated with cyclo. In the M2661 group, cyclo treatment resulted in an ILS of nonsurvivors (death delay). The effect of cyclo in M2661 on the percentage of cured animals was negligible and, consequently, the relapse rate reduction was minimal. In the LL group, cyclo delayed the reappearance of the disease and eradicated the residual tumor burden in some of the animals that would otherwise have been surgical failures. A host tumor cell interaction might have helped in eventually sterilizing the nearly cured animals and, since our LL tumor line is weakly immunogenic, this possibility cannot be ruled out.

By reducing the micrometastastic tumor load at the time of systemic treatment with cyclo, ie, by administering cyclo preoperatively to M2661-bearing mice, we obtained a significant difference between the survival curves. It should be noted that, for the LL, preoperative treatment is less effective than postoperative chemotherapy (table 1).

Instead of applying cyclo treatment at an earlier time point, ie, preoperatively, M2661 experiments were designed in which mice with small primary tumors (eg, T1 tumors) with minimal tumor loads after surgery were treated with cyclo (table 2). The nearly cured T1 M2661-bearing mice were cured by cyclo adjuvant chemotherapy in a statistically significantly higher proportion than those animals operated on for T4 tumors. This is

a common observation in a variety of experimental metastasizing tumor systems in which cures are achieved by adjuvant chemotherapy if the median residual burden is low and few animals represent near cures (5–7).

We applied two equations as described earlier to the survival data and calculated the cyclo-induced effect on survival time separately from the effect on relapse rate. The data of the M2661 and the LL shown in table 2 indicate that the ILS as a result of cyclo treatment is greater in the M2661 than in the LL group and is almost constant in all of the T categories. The induced relapse rate reduction, however, is more pronounced in the LL group. The relapse rate reduction in both tumor lines increases with decreasing tumor load and corresponds to the percent of animals nearly cured by surgery only. The effects on death delay and relapse rate reduction influence the shape of the survival curves. The curves for mice inoculated with M2661 cells and subjected to surgery only seemed to lag behind those for mice treated with cyclo adjuvant therapy; the curves later almost converged (fig 2). The survival curve for LL-bearing mice treated with adjuvant therapy continued to remain above that for the surgical controls.

Mice with primary tumors were treated with cyclo in the same dosages as administered in the adjuvant therapy experiments. As shown in table 3, the earlier the cyclo was administered after tumor cell inoculation, the greater was the observed growth delay. In both tumor types, the local cure rate was decreased when the cyclo treatment was delayed. Note, however, the difference in growth delay after early treatment in both tumor lines. Although the local cure rate in the M2661 is lower in comparison with LL, the opposite is observed for the growth delay. As shown in table 4, mice with very large foot pad tumors were treated with cyclo and no effect on the growth curve of either tumor was observed. This lack of volume response in very large tumors, however, does not imply that cells are not sterilized locally. Tumor volume response determinations and growth delay calculations are indirect parameters of tumor cell kill, and, in general, the larger the size of the tumor, the less the volume response corresponds with actual log cell kill. The observed tumor size-dependent effectiveness of cyclo has been described in the literature (8–12). In our model, however, we were able to demonstrate in individual animals that a lack of primary tumor response does not automatically imply that the concomitantly present micrometastases also do not respond. According to the data presented in table 4, the lack of a volume response was not predictive for the ILS and, consequently, the prediction based on the measurable foot pad LL was a false-negative one. The data obtained from the M2661 cell line might have led to a false-positive interpretation (tables 1 and 3).

In clinical management, tumor sensitivity and tumor resistance to chemotherapy can be established only after therapy has been applied. The obtained tumor response, eg, of skin metastasis, enables the clinician to decide on whether to continue effective or to stop ineffective cytostatic treatment. This is in contrast with adjuvant chemotherapy, ie, the treatment of micrometastasis, where direct evaluation of tumor response is impossible. Consequently, the chance of starting ineffective postoperative therapy can be high. As demonstrated by our data, experimental tumors may differ in their response to adjuvant treatment. Small microscopic foci can be more or less responsive to the same treatment as are solid tumors (LL and M2661). If this lack of uniformity in response also occurs in human tumors, new strategies should be developed to select the optimal adjuvant drug for each tumor type and/or cancer patient individually (13).

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Modification by Nitrobenzylthioinosine-5′-Monophosphate of Pseudoisocytidine Pharmacokinetics in Mice and Rats Through Inhibition of Membrane Transport[1,2,3]

Norbert Kolassa,[4,5] Alan R. P. Paterson,[5,*] and Ting-Chao Chou[6,7]

In isolated, perfused mouse livers, initial rates of uptake of [2-^{14}C]pseudoisocytidine (PIC), measured during the first 15 secs of perfusion were markedly reduced when the perfusion medium contained 5×10^{-6} M nitrobenzylthioinosine (NBMPR), a potent inhibitor of nucleoside transport. A similar inhibition of PIC uptake occurred when mice were treated with NBMPR-P (the 5′-monophosphate of NBMPR) at doses > 0.2 mg/kg ip injected 30 mins prior to the liver perfusion assay. However, in vivo studies showed that a late effect of NBMPR-P was enhancement in PIC levels in liver and other tissues in mice and rats, relative to levels in animals that had not received NBMPR-P. Increases in incorporation of PIC into RNA reflected the NBMPR-P-induced increases in tissue levels of PIC. NBMPR-P and other inhibitors of nucleoside transport may have therapeutic applications in manipulation of the pharmacokinetic behavior and toxicity of nucleoside drugs. [Cancer Treat Rep 67:51–58, 1983]

Pseudoisocytidine (PIC), synthesized by Chu et al (1), is a C-nucleoside analog of cytidine (fig 1) that is not subject to enzymatic cleavage of the glycosidic bond, nor to enzymatic deamination (2,3). Following iv injection in rats, > 90% of the administered PIC was excreted unchanged in urine during a 24-hr period (4). PIC shows therapeutic activity toward various lines of mouse leukemia, both sensitive and resistant to arabinosylcytidine (2,3). The antiproliferative activity of PIC toward cultured leukemic cells was blocked by cytidine but not by deoxycytidine (2). The incorporation of PIC into acid-soluble nucleotides and into RNA and DNA fractions in neoplastic cells was competitively inhibited by cytidine (3).

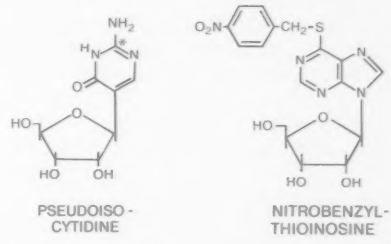

FIGURE 1.—Structures of PIC (pseudoisocytidine) and NBMPR (nitrobenzylthioinosine). Asterisk indicates labeled position in ^{14}C-PIC.

Hepatic injury is a prominent toxic effect of PIC in rats, cats, dogs, and monkeys (F. S. Philips, S. S. Sternberg, and P. M. Vidal, unpublished observation). The hepatotoxicity of PIC in rats correlated with the accumulation of this agent in the livers (4). Acute hepatocellular damage has also been observed in man

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[3]Preliminary reports from this investigation have appeared in the Proceedings of the American Association for Cancer Research 21:284, 1980, and in Federation Proceedings 39:536, 1980.

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(5,6). The aim of the present study was to determine whether (*a*) the tissue distribution and pharmacokinetic behavior of PIC would be modified by a potent inhibitor of nucleoside transport, and (*b*) such modulation might be used to decrease the hepatotoxicity of PIC.

We have reported recently that the cellular uptake[8] of cytidine during perfusion of the isolated liver in mice was almost completely blocked by either (*a*) inclusion of the nucleoside transport inhibitor nitrobenzylthioinosine (NBMPR, fig 1) in the perfusion medium, or (*b*) pretreatment of the mice with NBMPR, the 5′-monophosphate of NBMPR (7). These results suggested that cytidine entry into liver cells is mediated by a NBMPR-P-sensitive nucleoside transport mechanism, the existence of which has been demonstrated in various cell types, including erythrocytes and HeLa cells (8–10). The present report concerns the influence of NBMPR and NBMPR-P on the cellular uptake of PIC in isolated, perfused livers of mice, and on the pharmacokinetic behavior of PIC in rats and mice.

MATERIALS AND METHODS

PIC (1) and [2-^{14}C]PIC (2.75 mCi/mmol) (3) were provided by Dr. J. J. Fox (Memorial Sloan-Kettering Cancer Center, Rye, NY). [Methoxy-^{3}H]inulin (167 μCi/mg) was purchased from New England Nuclear Corp (Boston, MA), [carboxyl-^{14}C]inulin (2.4 μCi/mg) was purchased from Amersham Corp (Arlington Heights, IL), and [5-^{3}H]cytidine (6.25 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA). NBMPR was synthesized, as described previously (11), starting with 6-thioinosine provided by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). NBMPR-P was prepared as the disodium salt (12) and dosages are specified in terms of that salt.

Liver perfusion.—Male (C57BL/10J × DBA/2J)F_1 (B10D2F_1) mice (22–27 g) were obtained from the Health Sciences Small Animal Program, University of Alberta. The animals were starved overnight and anesthetized with sodium pentobarbital (70 mg/kg), which was injected ip about 15 mins before liver perfusion procedures began. The perfusion medium, a bicarbonate-buffered salt solution (13), containing 5 mM glucose and 10 units/ml of heparin, was filtered (filter pore size, 0.22 μm, Millipore Corp, Bedford, MA) and equilibrated with 5% CO_2 in O_2. The method of Hems et al (14) for the perfusion of rat liver was applied to mouse liver with minor modifications (7). Livers were perfused at room temperature (22°C) via the portal vein; a perfusion rate of 3.8 ml/min resulted in a perfusion pressure of 120–180 mm of H_2O. Free outflow of perfusate was assured by severance of the hepatic veins. The perfusion medium was delivered by an infusion pump (Model 940, Harvard Apparatus, Millis, MA) equipped with two syringes. Syringe outlets were connected to a four-way valve, which allowed (*a*) livers to be perfused with medium from either syringe, and (*b*) a virtually instantaneous change from one to the other. The other two connections of the four-way valve led to the perfusion cannula and to waste. After an initial 3-min period of perfusion with nucleoside-free medium delivered from one syringe, the flow through the liver was switched to medium (from the other syringe) containing either (*a*) ^{14}C-PIC together with ^{3}H-inulin, or (*b*) ^{3}H-cytidine and ^{14}C-inulin. To end perfusion intervals, livers were quickly excised, blotted on filter paper, and weighed; liver wet weights ranged between 0.75 and 1.05 g, and the mean ± SD of dry weight:wet weight ratios was 0.34 ± 0.02 (n = 81).

Liver content of labeled perfusion substrates.—Livers were divided into two roughly equal samples that were dried overnight at about 110°C, prior to assay of their ^{3}H and ^{14}C content. After samples were combusted in a Packard Model 306 Sample Oxidizer, the ^{3}H and the ^{14}C contents of each sample were determined in the separately collected combustion products, water and carbon dioxide. Inulin and nucleoside equivalent[9] concentrations in liver samples were calculated from (*a*) the ^{3}H content, the ^{14}C content, and the wet weight of each tissue sample, and (*b*) the concentrations of labeled inulin and ^{14}C-PIC (or ^{3}H-cytidine) in the perfusion medium. Curves in the figures were fitted to the data by eye.

Cell and tissue distribution studies.—B10D2F_1 mice and CD rats were purchased from Charles River Breeding Laboratories (Wilmington, MA). The P815 leukemia was maintained by weekly transplantation (10^6 cells ip) in B10D2F_1 mice; experiments with leukemic mice were carried out on the fifth day after transplantation. Tissue levels of ^{14}C-PIC were estimated from the ^{14}C content of the acid-soluble fraction. At different times after administration of labeled nucleoside to mice or rats, animals were killed by cervical dislocation or by bleeding under ethyl ether anesthesia. Tissue, blood, or plasma samples were weighed and homogenized with five volumes of 10% ice-cold perchloric acid. The homogenates were centrifuged at 2°C (15,000 × *g* for 15 mins) to obtain the acid-soluble fractions that were assayed for radioactivity with a Packard Tri-Carb Model 3775 liquid scintillation spectrometer using Hydrofluor scintillation fluid (National Diagnostics, Somerville, NJ). Tis-

[8]"Uptake" refers to the appearance of a permeant and metabolites thereof in cells or in the intracellular compartment of a tissue. "Transport" refers to the carrier-mediated passage of permeant across the plasma membrane.

[9]"Nucleoside equivalents" is used to represent in molar terms the cellular content of a nucleoside permeant plus metabolites formed therefrom.

sue levels of compounds (in microgram equivalents of nucleoside per gram or milliliter) were calculated from the specific radioactivity of ^{14}C-PIC. Incorporation of ^{14}C-PIC into RNA and DNA was measured by a method that was previously described (3).

RESULTS

Time Course of PIC Uptake in Perfused Mouse Liver and Inhibition by NBMPR

The experiment shown in figure 2 compared the time course of PIC uptake by perfused mouse liver to that of cytidine uptake; permeant concentrations in the perfusion medium were 10^{-5} M. The time courses of hepatic uptake of both nucleosides were approximately linear during the first 15-sec interval after initiating flow of the permeant-containing medium through the liver. Back extrapolation of these time courses yielded time-zero values for tissue concentrations of PIC or cytidine that approximated the inulin space (mean ± SD, 97 ± 11 μl/g wet weight; n = 51). Accordingly, it would appear that time-zero values for the nucleoside content of perfused livers obtained in this way represent the extracellular space. The ^{14}C-PIC and the ^{3}H-cytidine (permeant plus metabolites) found in the perfused livers in excess of those found in the inulin space represent permeant molecules which entered liver cells, initial rates of nucleoside uptake determined in this way were considered to represent unidirectional fluxes into liver cells. The effects of the inhibitor of nucleoside transport, NBMPR, on rates of hepatocellular uptake of PIC and cytidine (*see* below) support this interpretation.

The initial rate of PIC uptake by liver cells was about one-third that of cytidine under the conditions of these experiments. Inclusion of 5 μM NBMPR in the perfusion medium reduced cellular uptake rates of cytidine and PIC to about 2% and 15% of their respective uninhibited values. The NBMPR inhibitory effect indicates that the nucleoside transport mechanism participates in the hepatocellular uptake of both nucleosides and the extent of that inhibition argues that the observed fluxes approximated rates of inward transport of PIC. The NBMPR-insensitive portions of PIC fluxes (rates of PIC uptake in the presence of 5 μM NBMPR) were about twice those of cytidine.

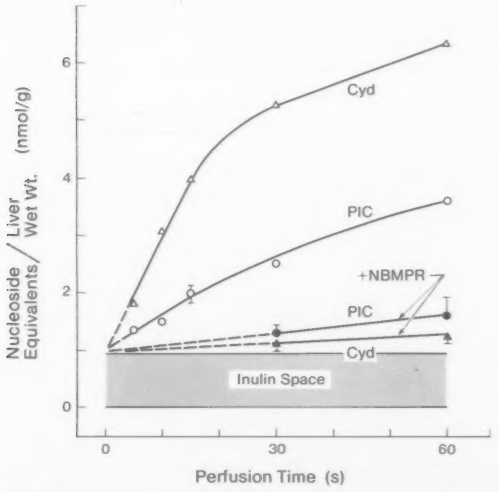

FIGURE 2.—Time courses of PIC and cytidine (Cyd) uptake by the isolated, perfused mouse liver. Livers were perfused with medium containing (*a*) 10^{-5} M ^{14}C-PIC and 0.6 mg/L of ^{3}H-inulin, or (*b*) 10^{-5} M ^{3}H-cytidine and 20 mg/L of ^{14}C-inulin for the indicated time intervals. The accumulation of ^{14}C-PIC (○,●) or ^{3}H-cytidine (Δ,▲) in the liver was assayed under control conditions (○,Δ) or when the perfusion medium contained 5 μM NBMPR (●,▲). Symbols represent the mean of 2–4 determinations; error bars (SD) are drawn in instances of ≥ 3 determinations.

Inhibition of PIC Uptake in Perfused Mouse Liver by NBMPR-P

Inhibition of PIC uptake was observed in perfused livers of mice that had received an ip injection of NBMPR-P about 30 mins prior to the perfusion assay (fig 3). NBMPR-P, formulated as the triethylammonium or the sodium salts, has been used in in vivo experiments as a readily soluble form of NBMPR (12). The latter is evidently formed by phosphohydrolase activity before interaction with the nucleoside trans-

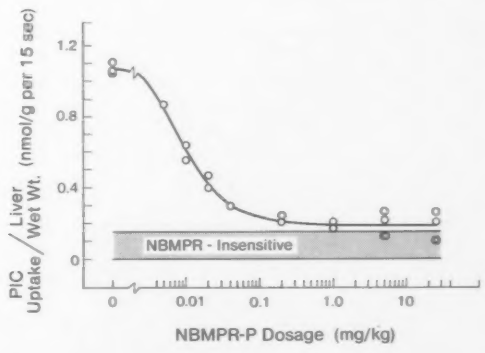

FIGURE 3.—Inhibition of PIC uptake by perfused mouse livers resulting from prior treatment with NBMPR-P. Mice were injected ip with the specified doses of NBMPR-P 30 mins prior to the perfusion assay of hepatic PIC uptake. Livers were perfused for 15 secs with medium containing 10^{-5} M ^{14}C-PIC and 0.6 mg/L of ^{3}H-inulin. Measured value for the ^{14}C content (PIC + metabolites) of each liver was reduced by the PIC present in the inulin space of that liver. The shaded area represents the NBMPR-insensitive component of PIC entry into liver cells as estimated from fig 2. Symbols represent single determinations.

porter (15). With NBMPR-P doses > 0.2 mg/kg, the NBMPR-sensitive component of PIC entry into liver cells was almost completely eliminated. PIC uptake was inhibited by about 50% in perfused livers from mice treated with 0.01 mg/kg of NBMPR-P.

Prolongation of the time interval between NBMPR-P injection and liver perfusion diminished the NBMPR-P inhibitory effect on hepatic uptake of PIC. The NBMPR-sensitive component of the PIC flux into liver (from perfusion medium containing 10^{-5} M PIC) was reduced by 85%, 55%, 40%, and 30% when time intervals between NBMPR-P (0.04 mg/kg) injection and the perfusion assay were 30, 60, 120, and 360 mins, respectively.

Inhibition of PIC Uptake in Perfused Mouse Liver by Cytidine

Inclusion of cytidine in the perfusion medium decreased the rate of PIC entry into liver cells during perfusion (fig 4). Rates of PIC uptake approached those of the NBMPR-insensitive component of PIC uptake when cytidine was included in the perfusion medium at concentrations > 3×10^{-4} M. At 10^{-4} M cytidine, the NBMPR-sensitive PIC flux (difference between the total hepatic uptake of PIC and the NBMPR-insensitive component of uptake during 15 secs of perfusion) was inhibited by about 50%.

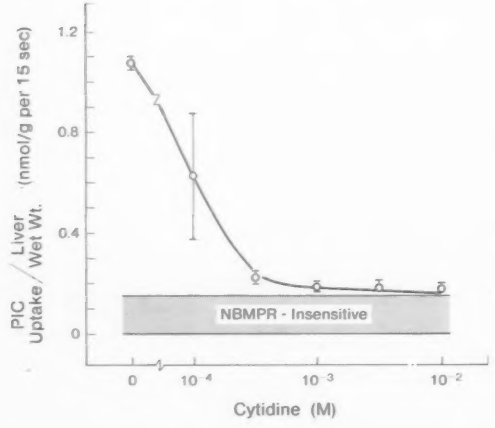

FIGURE 4.—Inhibition of cytidine of PIC uptake by mouse liver during perfusion. Livers were perfused for 15 secs with medium containing 10^{-5} M ^{14}C-PIC and 0.6 mg/L of ^{3}H-inulin + cytidine at the concentrations specified. The measured value for the ^{14}C-content (PIC + metabolites) of each liver was reduced by the PIC present in the inulin space of that liver. Shaded area represents the NBMPR-insensitive component of PIC entry into liver cells as estimated from fig 2. Symbols and error bars represent the mean ± SD of 3 determinations.

Effect of Dipyridamole and Cytidine on Uptake of PIC by Mouse Liver In Vivo

Treatment of mice with cytidine (200 mg/kg ip) 10 mins before the iv administration of ^{14}C-PIC (10 mg/kg) did not change PIC levels in plasma or tissues measured 10 mins after PIC administration. However, ip treatment with dipyridamole, an inhibitor of nucleoside transport (16), at a dose of 25 mg/kg decreased PIC levels in plasma and muscle and increased PIC levels in liver (table 1). Because of these findings, effects of the more potent inhibitor of nucleoside transport, NBMPR-P, on PIC levels in vivo were investigated.

Effect of NBMPR-P Pharmacokinetics of PIC in Mice and Rats

Ten minutes after iv injection of rats with ^{14}C-PIC at a dose of 10 mg/kg, levels of PIC in liver and spleen were significantly higher than in plasma. When rats were treated ip with NBMPR-P at 5 mg/kg 15 mins prior to iv administration of ^{14}C-PIC, levels of the latter in plasma and small intestine were not significantly affected by NBMPR-P. However, the levels of PIC in the spleen were slightly increased and those in the liver were markedly increased during the observation period, 10–120 mins after PIC administration (fig 5).

The incorporation of ^{14}C-PIC into RNA and DNA of tissues from rats with and without prior treatment with NBMPR-P was measured in the same experiment (table 2). More radioactivity was incorporated into RNA than into DNA in liver, spleen, and small intestine ($P \leq 0.01$). NBMPR-P treatment increased the incorporation of ^{14}C-PIC radioactivity into liver RNA during a 2-hr interval ($P \leq 0.1$).

Treatment of mice by the sc route with NBMPR-P (25 mg/kg) 10 mins before the sc administration of ^{14}C-PIC (5 mg/kg) caused changes in the time courses of PIC uptake and in PIC retention in several tissues (fig 6). PIC levels in spleen, liver, small intestine, and whole blood were initially lower in NBMPR-P-treated mice than in saline-treated control mice, but the maximum ^{14}C levels eventually attained in spleen and liver in NBMPR-P-treated mice were higher than in saline-treated animals. Maximum ^{14}C contents in liver, spleen, and small intestine were attained about 1–1.5 hrs after administration of ^{14}C-PIC. Net losses of ^{14}C from spleen, liver, and small intestine occurred between 0.5 and 2.0 hrs after injection of ^{14}C-PIC in mice untreated with NBMPR-P but did not occur in treated mice. Thus, differences between NBMPR-P-treated and untreated mice with respect to the ^{14}C-PIC uptake-retention patterns in the three tissues were such that the PIC equivalent contents of the tissues in treated mice were about twice those in untreated mice 2 hrs after PIC administration (fig 6). The blood kinetics of ^{14}C-PIC probably

TABLE 1.—Influence of prior treatment with cytidine or dipyridamole on tissue distribution of ^{14}C-PIC in B10D2F$_1$ mice*

Prior treatment	^{14}C-PIC content (nmols/g)			
	Plasma†	Liver	Spleen	Muscle
Saline	35.3, 34.7	113.0, 107.1	64.5, 54.3	29.2, 24.9
Dipyridamole (25 mg/kg)	27.1, 25.4	159.3, 157.3	70.2, 62.1	20.9, 17.7
Cytidine (200 mg/kg)	35.6, 35.4	114.3, 113.9	45.4, 45.3	26.4, 24.6

*Mice were killed and tissues sampled 10 mins after iv administration of ^{14}C-PIC at 10 mg/kg (41 μmols/kg). The 2 animals in each treatment group received dipyridamole, cytidine, or saline by the ip route 10 mins before PIC.
†nmols/ml.

TABLE 2.—Incorporation of ^{14}C-PIC into RNA and DNA in tissues of CD rats with or without prior administration of NBMPR-P*

Time (mins)	Experiment (± NBMPR-P)	^{14}C-PIC incorporation (pmols/g)					
		Liver		Spleen		Small intestine	
		RNA	DNA	RNA	DNA	RNA	DNA
10	−	114 ± 3	47 ± 10	118 ± 33	25 ± 16	81 ± 9	21 ± 5
	+	123 ± 8	47 ± 5	140 ± 42	20 ± 4	96 ± 6	11 ± 10
20	−	145 ± 24	55 ± 3	365 ± 50	96 ± 65	105 ± 10	42 ± 10
	+	151 ± 9	44 ± 13	414 ± 179	102 ± 65	84 ± 30	35 ± 19
30	−	164 ± 13	65 ± 18	623 ± 70	58 ± 19	220 ± 62	149 ± 19
	+	184 ± 4	64 ± 17	489 ± 53	83 ± 4	211 ± 37	121 ± 14
120	−	203 ± 1	55 ± 7	1399 ± 135	494 ± 321	308 ± 151	212 ± 125
	+	345 ± 47	101 ± 22	1354 ± 111	440 ± 115	564 ± 205	216 ± 86

*NBMPR-P (5 mg/kg ip) was administered 15 mins prior to ^{14}C-PIC (10 mg [5 μCi]/kg iv). Animals were killed and tissues sampled at the indicated times after administration of ^{14}C-PIC. Values = mean from 2 (± deviation) or 3 (± SE) animals. Incorporation of ^{14}C into RNA was greater than into DNA ($P \leq 0.01$) in all 3 organs with or without prior administration of NBMPR-P. After 120 mins, the incorporation of ^{14}C-PIC into liver RNA was greater in NBMPR-P-treated animals than in untreated animals ($P \leq 0.1$).

reflect (*a*) NBMPR-sensitive, transporter-mediated uptake of PIC by erythrocytes without intracellular phosphorylation of the analog, and (*b*) NBMPR inhibition of absorption of sc injected ^{14}C-PIC into the blood stream.

Figure 7 presents data from an experiment similar to that of figure 6, except that mice bearing the P815 leukemia were employed, and leukemic cells (obtained from ascitic fluid) were assayed for ^{14}C content at various times after sc administration of ^{14}C-PIC. Levels of ^{14}C-PIC were higher in the leukemic cells from NBMPR-P-treated mice than in cells from untreated mice. The experiments of figures 6 and 7 demonstrate that prior treatment with NBMPR-P changed the kinetics of PIC incorporation in cells and tissues in mice, thus markedly increasing the retention of PIC (and presumably of PIC metabolites) in spleen, liver, small intestine, and leukemic cells.

Because NBMPR is known to inhibit nucleoside transporter-mediated efflux of nucleoside permeants (8,17), NBMPR inhibition of PIC efflux was considered to be a possible basis for the enhancement of cellular retention of PIC equivalents that resulted from NBMPR-P treatment of mice (figs 6 and 7). This possibility was tested in the experiment shown in figure 8 in which time courses of PIC efflux from P815 cells loaded with ^{14}C-PIC were measured with a rapid sampling technique, centrifugal pelleting of cells under oil (18). When centrifuge pellets of PIC-loaded cells were rapidly resuspended in PIC-free medium, both the rate and extent of PIC efflux were reduced when the medium contained 5 μM NBMPR. Similarly, when cells were exposed simultaneously to ^{14}C-PIC and NBMPR (5 μM), the rate and extent of PIC uptake were much reduced by NBMPR relative to those in cells exposed to ^{14}C-PIC alone. The rate of ^{14}C-PIC uptake by these cells at 2°C was much lower than that at 22°C.

DISCUSSION

NBMPR and certain other S^6-derivatives of 6-thioinosine and 6-thioguanosine have been recognized as potent, specific inhibitors of nucleoside transport in various experimental systems (11,18). Inclusion of 5 μM NBMPR in the perfusion medium also diminished rates of PIC entry into perfused livers (fig 2). Inhibition of PIC influx was obtained with high concentrations of

cytidine (fig 4), which has been shown to enter liver cells via an NBMPR-sensitive transport mechanism (7). Both facts, the inhibition by NBMPR and the competition by cytidine, suggest that the major portion of PIC permeation through the plasma membrane of liver cells is carrier-mediated.

The NBMPR-sensitive portion of PIC entry into perfused liver was significantly smaller than that of cytidine when the concentrations of both permeants in the perfusion medium were 10^{-5} M (fig 2). Assuming that the same transport mechanism mediates the entry of both nucleosides into liver cells, this result suggests that PIC has a lower affinity than cytidine for that mechanism. The notion of a common mechanism is supported by the observation that high concentrations of cytidine in the perfusion medium reduced rates of PIC entry into liver cells to values similar to those of the NBMPR-insensitive component of PIC influx. An attempt to use a high dose of cytidine in vivo to influence the uptake of PIC in mouse tissues was not successful (table 1), probably because of extensive tissue distribution and metabolism of cytidine in vivo.

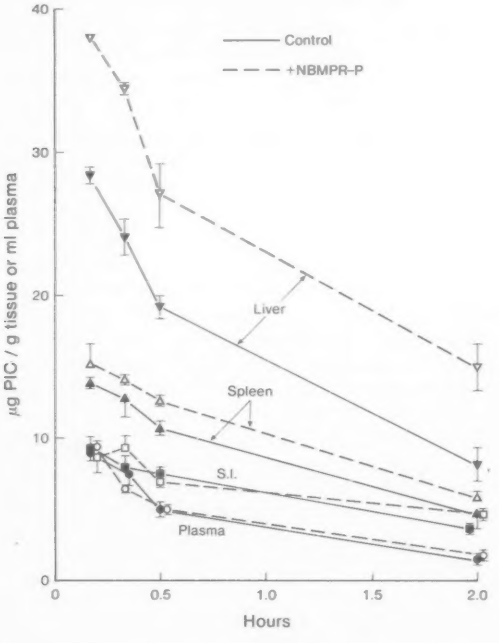

FIGURE 5.—Effect of NBMPR-P on distribution of ^{14}C-PIC in CD rats. NBMPR-P (5 mg/kg) was given by ip injection 15 mins prior to the iv injection of ^{14}C-PIC (10 mg [5 μCi]/kg; 41 μmols/kg). Animals were killed and tissues sampled at the indicated times after administration of ^{14}C-PIC. Data plotted are tissue contents of ^{14}C expressed as microgram equivalents of PIC and are the mean from 2 (± deviation) or 3 (± SE) animals. S.I. = small intestine.

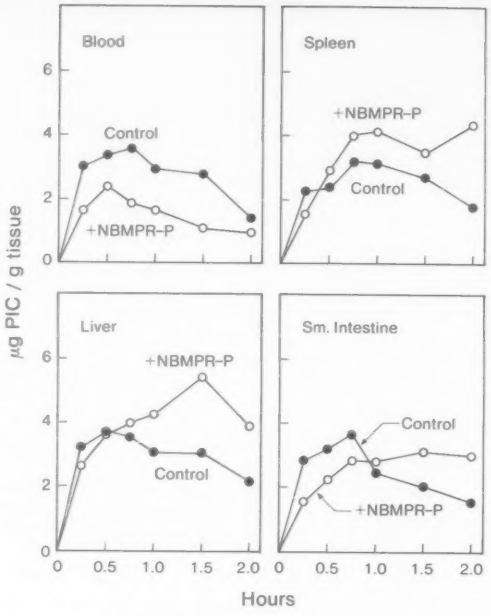

FIGURE 6.—Effect of NBMPR-P on distribution of ^{14}C-PIC in B10D2F$_1$ mice. Saline (control) or NBMPR-P (25 mg/kg) was given by ip injection 10 mins prior to the sc injection of ^{14}C-PIC (10 mg [5 μCi]/kg, 41 μmols/kg). Animals were killed and tissues sampled at the times indicated after administration of ^{14}C-PIC. Data plotted are tissue contents of ^{14}C expressed as microgram equivalents of PIC and are the mean obtained with 2 or 3 animals.

Treatment of mice with NBMPR-P at 0.01 mg/kg reduced the NBMPR-sensitive component of PIC entry into perfused livers by about 50% (fig 3). This result suggested that NBMPR-P might reduce the hepatotoxicity of PIC. However, protection against PIC toxicity was not apparent in experiments in which hepatic injury was monitored by SGPT activity. Liver damage was not alleviated by treatment with NBMPR-P at doses of 25 or 5 mg/kg in rats (single dose of PIC at 300 mg/kg ip) or cats[10] (single dose of PIC at 25 mg/m^2 iv), as judged by failure of that treatment to reduce the PIC-induced elevations in SGPT levels or to prevent death in these animals (data not shown). In those experiments, NBMPR-P was administered ip 30 mins before PIC. These findings appear to agree with other observations presented here indicating that, while NBMPR-P treatment of mice reduced the rate at which PIC initially appeared in liver, the hepatic content of PIC and metabolites eventually surpassed that in livers

[10]We are grateful to Dr. D. C. Secord, Health Sciences Large Animal Center, University of Alberta, for his help with these experiments.

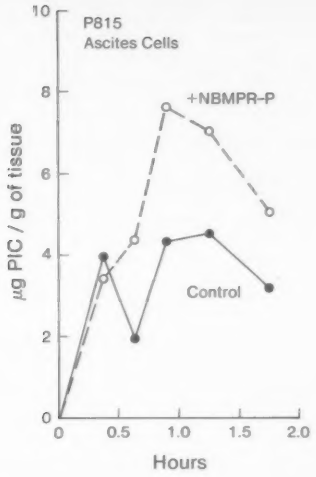

FIGURE 7.—Effect of NBMPR-P on retention of ^{14}C-PIC by P815 leukemia cells in B10D2F$_1$ mice. On the 5th day after implantation with 10^6 P815 leukemia cells, mice received sc saline (controls) or NBMPR-P (25 mg/kg) given 10 mins before the sc injection of ^{14}C-PIC (10 mg [5 μCi]/kg; 41 μmols/kg). At the times indicated after administration of ^{14}C-PIC, animals were killed and leukemia cells were collected from ascitic fluids. Acid-soluble extracts of the latter were assayed for ^{14}C content and these data are given in the fig as microgram equivalents of PIC. Reported is the mean obtained with 2 or 3 animals.

of mice that had not received prior treatment with NBMPR-P (compare with fig 6).

The inhibitory effects of NBMPR on nucleoside transport in cell suspensions (18) and in perfused liver (7) did not forecast the effects of NBMPR-P on the in vivo distribution of PIC. Following iv administration of PIC to rats, hepatic drug levels were increased significantly by prior treatment with NBMPR-P over those in control animals at the time points chosen for analysis (fig 5). Evidently, determinants of the in vivo hepatic uptake of PIC other than the activity of the liver nucleoside transport mechanism are also influenced by NBMPR-P pretreatment in ways which are not yet understood. For example, NBMPR-P inhibition of mediated entry of PIC into tissues other than liver may have influenced PIC plasma levels during the early distribution phase, and the tissue distribution of PIC under that condition may be determined by NBMPR-insensitive permeation. This interpretation is supported by the observation that treatment of mice with dipyridamole, a potent inhibitor of nucleoside transport, elicited increases in the hepatic PIC burden similar to those which followed NBMPR-P treatment and, at the same time, inhibited PIC uptake by skeletal muscle (table 1).

Earlier studies with P815 cells (3) indicated that chemotherapeutic effects of PIC correlated with tissue levels of PIC triphosphate and with amounts of PIC incorporated into RNA. The present study showed that NBMPR-P increased PIC incorporation into the RNA of rat tissues (table 2). A preliminary study has indicated that incorporation of PIC into RNA of P815 leukemic cells in vivo was also increased by NBMPR-P (T.-C. Chou and L.-I. Chen, unpublished observations).

The experiments reported here demonstrate a substantial influence of prior treatment with NBMPR-P on the kinetics of ^{14}C-PIC uptake in several mouse tissues and show that an initial inhibition of PIC uptake was followed by cellular retention of PIC or its metabolites. It is possible that NBMPR inhibition of transporter-mediated efflux of PIC is involved in this apparent retentive effect. We are unable to account for the late appearance of this effect. NBMPR-P-induced retention of PIC in tissues may be a contributing factor in the NBMPR-P enhancement of PIC toxicity reported here. The possible effect of NBMPR-P on renal clearance of PIC and the subsequent modification of pharmacokinetics and toxicity remain to be studied.

This enhancement by NBMPR-P of the PIC content

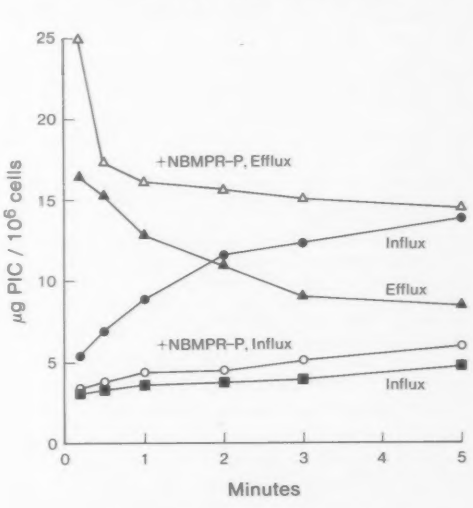

FIGURE 8.—Effect of NBMPR on influx and efflux of PIC in P815 leukemia cells. In the measurement of PIC influx, leukemia cells (obtained from pooled ascitic fluids) were suspended (9.2×10^7 cells/ml) in phosphate-buffered salts containing 100 μM ^{14}C-PIC with (○) and without (●,■) 5 μM NBMPR. Samples taken after graded intervals at 22°C (○,●) or at 2°C (■) were layered on oil and spun in an Eppendorf microcentrifuge to rapidly separate cells from medium. In the measurement of PIC efflux, P815 cells were "loaded" with ^{14}C-PIC by incubation for 30 mins with ^{14}C-PIC under the above conditions. Centrifuge pellets of PIC-loaded cells were rapidly resuspended in PIC-free medium with (△) or without (▲) NBMPR (control) at 22°C; samples taken from these suspensions at graded intervals after resuspension were separated into cell and medium fractions by oil-layer centrifugation.

of various tissues suggests that NBMPR-P or other inhibitors of nucleoside transport may have, under certain circumstances, applications in the use of nucleoside drugs employed in the treatment of neoplastic or virus diseases. The opposite effect of NBMPR-P on the distribution of several toxic nucleoside drugs in the mouse has been demonstrated, that is, reduction in toxic effects apparently due to reduced access of the toxic drugs to vital tissues. Protection of mice against the toxicity of the nucleoside drugs, nebularine, 3′-deazauridine, and arabinosylcytosine, has been obtained with the joint administration of NBMPR and NBMPR-P (19,20). In several instances, use of nucleoside analogs in combination with the transport inhibitor has increased the therapeutic index of the analogs (12,21). We suggest that the potent, tightly bound inhibitors of nucleoside transport may have applications in chemotherapy with nucleoside analogs by enabling some manipulation of the pharmacokinetics and tissue distribution of these drugs.

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Identical Efficacy of Methotrexate Regimens With N^5-Methyltetrahydrofolate Rescue or With Leucovorin Rescue for Treatment of L1210 Murine Leukemia[1,2,3]

P. F. Nixon* and L. Wilson[4]

A simple preparation of N^5-methyltetrahydrofolate (MF) has been developed to a stage that is satisfactory for animal cancer chemotherapy. The resultant material was 79% MF and 3% leucovorin (LV; N^5-formyltetrahydrofolate). Using this material, the efficacy of methotrexate regimens with MF rescue was compared to that of methotrexate regimens with LV rescue for treatment of L1210 leukemia in BDF_1 mice. In comparable regimens, the efficacy with MF was no less and no greater than that with LV. [Cancer Treat Rep 67:59–62, 1983]

N^5-Methyltetrahydrofolate (MF) has been shown to give protection from methotrexate (MTX) toxicity (1–3). Leucovorin (LV; N^5-formyltetrahydrofolate), the standard tetrahydrofolate used following MTX in a "rescue" regimen, is known to be metabolized rapidly to MF (4–7); hence, an MTX regimen with MF rescue should have an efficacy close to that of an MTX regimen with LV rescue. Since MF is chemically less stable than LV (7,8), its clinical use would be justified only if there were some special advantage of MF over LV. Such an advantage has been reported: ie, that MF will reverse the growth inhibition of normal cultured cells by MTX but *not* that of some cultured neoplastic cells, including L1210 (9). If this finding were to be extended to tumor-bearing animals, it would suggest that MTX continues to act against a sensitive neoplasm despite a plasma concentration of MF sufficient to protect normal host tissues from MTX toxicity. On the other hand, LV was claimed to reverse the effects of MTX on cultured neoplastic cells (9) as well as on cultured normal cells without distinction, an action already documented in animals (10).

In this report we describe a preparation of MF superior to that obtainable commercially. This preparation of MF is compared with LV in MTX regimens for the treatment of L1210 murine leukemia.

MATERIALS AND METHODS

BDF_1 mice (9.5–12.5 weeks of age; supplied by the Animal Breeding Establishment, University of Queensland) were given mouse food (Feed Supplies, Brisbane) and water ad libitum. The L1210 ascites leukemia was carried in serial transfer in BDF_1 mice with transfers on Day 7 following ip inoculation of 1×10^5 cells. For use in experiments, the cells were harvested on Day 7, diluted with physiologic saline, and inoculated sc into the right thigh. MTX was injected sc into the left thigh 53 hours after inoculation of the tumor. MF or LV was injected sc into the left side of the body either 15 hours after MTX or simultaneously with MTX. The animals were observed daily for tumors and deaths until Day 60; any that died without obvious tumor were autopsied. All toxic deaths occurred before Day 9 and after Day 4.

MTX and LV were supplied by Cyanamid Australia Pty, Ltd. MF was prepared by the following modification of the method of Chanarin and Perry (11). All solutions were nitrogen-gassed and the preparation was monitored by serially recorded spectra. LV (2.5 g) was suspended in 50 ml of 1 M HCl and stirred gently at 20°C. The resultant solution was cooled to 0°C, the pH was raised to 6 by the addition of 4 N NaOH, and 10

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[2]Supported by the Queensland Cancer Fund.
[3]Methotrexate and leucovorin were supplied by Dr. A. L. Hellestrand, Cyanamid Australia Pty, Ltd.
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mg of sodium borohydride was added. The pH was then raised to 8, and 5 g of solid $NaBH_4$ was added over 1 hour with the solution stirred under an N_2 blanket. Over the next hour, whenever the pH rose to > 9.5, it was adjusted down to 9.0. The pH was then slowly lowered to 7.8, 1.25 g of $CaCl_2.6H_2O$ was added, and the precipitated borate salts were removed. Two volumes of ethanol was added and the container stood 16 hours at –10°C. The resultant dense, fine, white precipitate of MF was collected, washed first in 75% then 100% ethanol, and stored up to 4 months at –80°C as a precipitate under ethanol containing 70 mM 2-mercaptoethanol. Solutions of MF for injection, made 16.6 mM in water containing 30 mM 2-mercaptoethanol, were adjusted to pH 7.8 and used within 2 hours. The concentration of solution was determined by recorded spectra (8). The purity of a preparation was determined by analytic ion-exchange column chromatography on DEAE-Trisacryl with other conditions as described previously (12).

RESULTS AND DISCUSSION

Preparation of MF

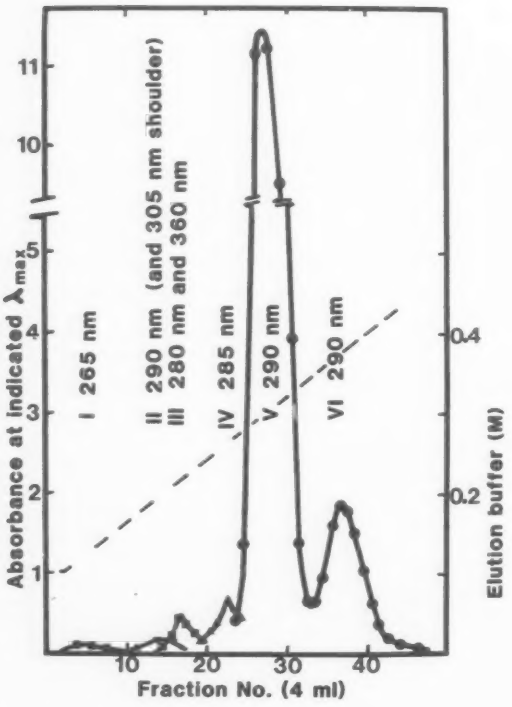

FIGURE 1.—Preparation of MF was analyzed by column chromatography as described in the Materials and Methods section. Elution was by potassium phosphate buffer, pH 6.0, of increasing concentration as indicated and containing 100 mM 2-mercaptoethanol. The UV absorbance spectrum of each 4-ml fraction was recorded and the absorbance of each was plotted at the λ_{max} for that fraction.

The concentration of MF in three preparations was measured from the UV absorbance spectra, and the yields were in the range of 61%–79% of the starting LV. The purity of a 4-month-old preparation was analyzed by column chromatography, and UV absorbance spectra were recorded for each fraction. Six peaks were separated (fig 1) and the quantity of material in each was estimated by integration and use of the published extinction coefficients (8) of those folate derivatives best identified by both the elution position (12) and the UV absorbance spectra. The major component (peak V) had a UV absorbance maximum at 290 nm, consistent with MF, and was 79% of the preparation. Peak VI had the same spectrum but was eluted later, consistent with 5-methyldihydrofolate, and was 14% of the preparation. Peak IV contained material having an absorbance maximum at 285 nm, consistent with LV, and was 3% of the preparation. Peak III was consistent with 5,10-methenyltetrahydrofolate (λ_{max} at 280 and 360 nm) and was 2% of the preparation. Peaks I and II were other minor components, each approximating 1% of the preparation.

This preparation of MF follows the principles outlined by Chanarin and Perry (11). However, they adopted conditions which would have achieved a pH value < 2 during the reduction and consequently required > 50 g of borohydride/1 g of LV. We have found that 2 g of borohydride/1 g of LV is sufficient, provided the pH is controlled in the range of 8–9.5. Moreover, the precipitation steps described here provide a preparation which is still lower in residual borate. The color of the MF

preparation, stored at –80°C as a precipitate, remained white for 4 months but then became off-white. A solution made up in 30 mM 2-mercaptoethanol and kept at 0°C remained colorless for 2 hours but thereafter became cream-colored then yellow-colored. Only colorless solutions of MF were used in the experiments described below, unlike the tan-colored preparation available commercially.

Efficacy of MTX With MF or LV in the Treatment of L1210 Leukemia

When MF was administered 15 hours after MTX (tables 1 and 2), the antitumor efficacy of the MTX was retained while the toxicity of higher doses of MTX was prevented. When the tumor inoculum was 200 cells, the results for MF were slightly poorer than those for LV, but there was little difference between the two when the inoculum was 2000 cells. These results held over a range of MTX doses varying from 63 to 500 mg/kg. Thus, the efficacy of regimens in which MTX was followed by delayed MF was not better than those in which MTX was followed by a delayed, equivalent dose of LV, a result similar to previously published findings (10,13).

The molecular weights of MTX, LV, and MF are very similar. Therefore, it is clear that a single dose of LV or MF can afford protection from the toxicity of MTX, even when the molar ratio MTX/LV or MTX/MF approaches 5/1. In these experiments, both the MF and the LV were racemic mixtures of the two stereoisomers, about carbon 6. In the case of LV, only the physiologic stereoisomer reverses MTX toxicity (3) and it is probable that that is true of MF also.

To test the claim that MF does not protect L1210 cells from MTX (9), we administered MTX and MF simultaneously to mice previously inoculated with L1210. The results (tables 1 and 2) indicated that simultaneous MF at a dose of 100 mg/kg abolished the efficacy of MTX at 63 mg/kg and significantly reduced the efficacy of MTX at doses up to 500 mg/kg. Thus, MF rescue did not differentiate between L1210 neoplastic cells and normal mouse tissues. Moreover, this result for MF is very similar to that previously obtained for MF (13) and for LV (10,13).

At the doses tested, there appeared to be no advantage in using MF over LV in regimens with MTX. Admittedly, only relatively low inoculations of L1210 were utilized in these experiments, and MF was used in a single dose at only two time intervals after MTX. Nevertheless, the results appear to be sufficiently conclusive to suggest that no other combination of MF with MTX would be superior to an equivalent combination of LV with MTX. Since storage and handling are more difficult for MF than for LV, since LV is metabolized

TABLE 1.—Efficacy of MTX regimens with LV or with MF in the treatment of L1210 murine leukemia (inoculum of 200 L1210 cells sc)

MTX dose (mg/kg)	No. of mice in group	No. of toxic deaths	Day of tumor death (No. of mice)*	Median survival time (days)†	Median increase in lifespan (%)†	No. of 60-day survivors
			No therapy controls			
0	20	—	14(2),15(4),16(8),17(3),18(2),19	16	—	0
			MTX alone‡			
63	10	0	17,18(3),19(2),20,28	19	19	2
125	10	0	18(2),19,21(2),22(2)	20	25	3
250	10	1	19,21,22(2)	21	31	5
500	10	5	18	17.5	9	4
			MTX then LV (100 mg/kg) 15 hrs later			
63	9	0	17(2),18,20(4),26,32	20	25	0
125	8	0	18,20(3),22,28,31,35	25	56	0
250	9	0	17,22,28,36	27.5	72	5
500	9	0	20(2),24(2)	23.5	47	5
			MTX then MF (100 mg/kg) 15 hrs later			
63	9	0	16(2),17,18(2),20,21,22,31	19	19	0
125	9	0	17,20(2),38	20	25	5
250	8	0	17,20(2),21,22	20	25	3
500	8	0	20(2),21(2),22	21	31	3
			MTX and MF (100 mg/kg) simultaneously			
63	10	0	14,15(5),16(3),19	15	0	0
125	8	0	16(2),18,19(3),20,22	19	19	0
250	11	0	16(3),17,18(4),19,21(2)	18	13	0
500	10	2	16,18(3),20,22,27	19	19	1

*Days, after tumor inoculation, on which mice died with palpable tumors. Nos. in parentheses = No. of mice that died, if > 1.
†Calculated for those mice that died due to tumor.
‡MTX was administered 53 hrs after tumor inoculation.

TABLE 2.—Efficacy of MTX regimens with LV or with MF in the treatment of L1210 murine leukemia (inoculum of 2000 cells sc)

MTX dose (mg/kg)	No. of mice in group	No. of toxic deaths	Day of tumor death (No. of mice)*	Median survival time (days)†	Median increase in lifespan (%)†	No. of 60-day survivors
			No therapy controls			
0	20	—	13(7),14(6),15(2),16(3),17(2)	14	—	0
			MTX alone‡			
63	10	0	15,16(2),17(2),18,19,20,21,22	17.5	25	0
125	9	0	18,19(3),24,26	19	36	3
250	11	0	17,18(2),19(4),20,21,23	19	36	1
500	10	7	—	—	—	3
			MTX then LV (100 mg/kg) 15 hrs later			
63	11	0	15(2),16(2),17(3),18(2),19,20	17	21	0
125	11	0	15,18,19,20,22	19	36	6
250	11	0	16(3),17(3),18(3),20	17	21	1
500	11	0	17,18(2),19,20,23,27,35	19.5	39	3
			MTX then MF (100 mg/kg) 15 hrs later			
63	11	0	15,16,17(3),18(3),19,21	17.5	25	1
125	11	0	16(2),17(3),18(3),19(2)	17.5	25	1
250	11	0	17,18(2),19(3),20(4)	19	36	1
500	11	0	16,17,18(2),19,20,22,24	18.5	32	3
			MTX and MF (100 mg/kg) simultaneously			
63	10	0	13,14(4),16(2),17(3)	16	14	0
125	8	0	14,15(3),16,17(2),21	15.5	11	0
250	10	1	15,16(4),17(3),18	16	14	0
500	10	2	16,17(3),18,19(2),20	17.5	25	0

*Days, after tumor inoculation, on which mice died with palpable tumors. Nos. in parentheses = No. of mice that died, if > 1.
†Calculated for those mice that died due to tumor.
‡MTX was administered 53 hrs after tumor inoculation.

rapidly in vivo to MF (4,7), and since these data show that MF is not superior to LV in a regimen against an MTX-sensitive tumor model, it is clear that delayed LV remains the tetrahydrofolate of choice for use in an MTX rescue regimen.

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Evidence of Possible Dose-Dependent Doxorubicin Plasma Kinetics in Man[1,2]

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The published plasma clearance profiles of doxorubicin in man (with differing liver status) have been analyzed further using a kinetic model instead of a linear regression model. At high (60 mg/m^2 and 45 mg/m^2) and intermediate (30 mg/m^2) doxorubicin dose levels, a triphasic drug clearance pattern is apparent. However, at a low dose level (15 mg/m^2), a biphasic profile can adequately describe the kinetics. This association of the kinetics with drug dose has been demonstrated in several ways, the simplest of which involves graphic overlaying of juxtaposed plasma clearances, and shows the emergence of a kinetic profile for low dose levels that is markedly different from that at high dose levels. At doses greater than about 45 mg/m^2, a probable saturation of response mechanisms is observed. It is not possible to interpret these results in terms of pure dose-dependent responses because of the varying health status of the individual subjects between and within experimental treatment. However, in light of our findings further investigation into the possibility of such an effect should be undertaken, particularly in view of its clinical ramifications. [Cancer Treat Rep 67:63–69, 1983]

Doxorubicin is an anthracycline antibiotic that is widely used in the treatment of cancers because of its broad spectrum of activity (1,2). The chemical (3), physicochemical (4), and biochemical (4–6) effects of doxorubicin have been reviewed, as have the distribution and metabolism of the drug (5). The primary mode of action of doxorubicin is thought to be at the DNA level (1,4), although many other receptor sites have been suggested, and this subject has also been reviewed in recent years (4,6,7). A complete understanding of the biologic effects of anthracyclines remains complicated by the variety of metabolites detected (5,8,9) and the lack of knowledge as to their cardiotoxic (5,10) or chemotherapeutic (2,4,5) roles.

In an effort to improve understanding of the behavior of these drugs, there have been many attempts to interpret the pharmacokinetic profile of anthracycline utilization in man (8,11–20). Unfortunately, characterization of these profiles has been complicated by the presence of metabolites, a factor not considered in all of the earlier investigations. These earlier studies suggested that the plasma disappearance in man, following iv administration, exhibited a biphasic pattern (13–15). More recently, however, triphasic profiles have been reported (8,16) and have been supported by Reich et al (18) who fitted a three-compartment model to doxorubicin plasma kinetics. In the study by Reich et al, doxorubicin was separated from its metabolites by thin-layer chromatography (TLC). This study therefore provides a good description of doxorubicin pharmacokinetics.

A complete and accurate description of anthracycline pharmacokinetics is clearly mandatory if anthracycline therapy is to advance. Although several pharmacokinetic studies of doxorubicin in man have used varying doses of drug (16,17,19), only the study of Bachur et al (16) presents a full analysis of a multiple dosing program. However, this study was based on a linear model which assumes that plasma drug level at time t is the sum of drug levels in different phases at that time (ie, drug kinetics are independent of drug level), viz:

$$Y_t = \Sigma\beta_j X_j t + \Sigma\beta_k X_k + \epsilon_t,$$

where Y_t = plasma drug level at time t, X_j = a dummy (0,1) variable defining the j'th response phase, t = time after drug administration, β_j = regression coefficient, and ϵ_t = error in the model.

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To gain further insight into the kinetics of these drugs, this linear model must be replaced by a kinetic model (21). During the course of developing a kinetic model of known doxorubicin plasma decay profiles in man (16), we noted that the kinetics appeared to be dependent on the doxorubicin dose level. We report here this apparent dose dependence, show that a triphasic profile exists at high dose levels, and demonstrate that this reduces to a biphasic profile at low drug doses.

MATERIALS AND METHODS

The subjects studies by Bachur et al (16) were patients admitted to the Baltimore Cancer Research Center (Baltimore, MD) for treatment of solid tumors and refractory acute lymphocytic leukemia. All had normal renal function. The doxorubicin dose, administered by iv infusion, was 100 μmols (60 mg)/m^2 of body surface area (12 patients; 16 studies). This was reduced to 45 mg/m^2 (eight patients), 30 mg/m^2 (nine patients; ten studies), and 15 mg/m^2 (four patients) on the basis of elevated BSP retention, increased bilirubin level, or prior evidence of severe marrow suppression by anticancer drugs, as outlined by Benjamin et al (22).

Blood samples were taken immediately prior to, and at intervals after, administration of the drug; the plasma fraction was collected and then analyzed by fluorometric assay and radioimmunoassay (RIA) as described by Bachur et al (16). Only the RIA-derived data were used for the present analysis, since RIA is considerably more specific than fluorometry, detects essentially only the intact nonmetabolized parent drug and the major metabolite adriamycinol (23), and is considerably less sensitive to all other currently known metabolites (13,16,23).

The computer modeling program, CONSAM (24), was used on a VAX 11/780 (Digital Equipment Corp) to simulate kinetic responses and to estimate parameters for the models. This is an interactive version of SAAM, the simulation analysis and modeling program developed by Berman et al (25).

To simulate kinetic responses, this program utilizes the concept of compartments joined by connecting pathways, quantified as kinetic parameters. For example, L (I,J) implies the presence of compartments I and J (conceivably discernable subspaces equilibrating with the drug at different rates), a connection between them, and a fractional clearance rate, L, of drug from compartment J to compartment I (ie, a rate constant). Further details of the operational units essential to this investigation are outlined in the legend to figure 1.

The program translates the kinetic parameters, and other constructs, into a system of differential equations and solves these to provide simulations of the drug response. When the simulation is inconsistent with the observed responses, the model structure or parameter values are deemed to be inappropriate. Alteration of the model structure is accomplished by deletion or insertion of kinetic parameters, while selection of a better set of parameters can be accomplished automatically by the program.

The data of Bachur et al (16) were fitted to the three-compartment model of Reich et al (18). This model was

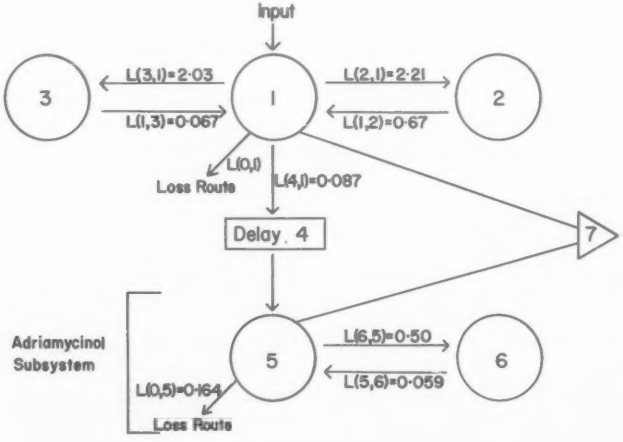

FIGURE 1.—3-compartment model and parameters used for simulating doxorubicin plasma disappearance profiles shown in fig 2. Arrows = drug transfer between compartments (circles). Nos. associated with arrows = rate constants (hr^{-1}). These values and the model configuration shown are those determined by Reich et al (18). Triangle (unit 7) = plasma where concentration of doxorubicin + adriamycinol was measured by RIA. Input represents a zero-order infusion of drug.

developed from the pharmacokinetic data obtained from four patients treated with doxorubicin at a dose of 60 mg/m^2. For this work, doxorubicin and adriamycinol were separated by TLC and quantitated by fluorometry.

RESULTS

Saturation Effect

The RIA data for average plasma levels of doxorubicin (including adriamycinol) of Bachur et al (16) have been plotted in figure 2. The curves were juxtaposed by scaling all data with respect to the value obtained for the first data point (0.5 hour) of the highest doxorubicin dose administered (60 mg/m^2). The scaling factors used were 1.0, 1.1916, 1.629, and 2.6375 for the 60-, 45-, 30-, and 15-mg/m^2 dose levels, respectively. Scaling by an unweighted least-squares adjustment procedure involving all data points resulted in virtually unaltered estimates of these factors.

The average responses of the two higher dose levels (60 mg/m^2 and 45 mg/m^2) were indistinguishable and were therefore combined in figure 2. However, this response is inconsistent with that for the lowest dose level (15 mg/m^2), with a transition at the intermediate dose (30 mg/m^2) being evident. We therefore consider this as indicative of a possible thresholding or saturation effect, with identical drug kinetics being evident only at a dose level > 45 mg/m^2. Although relatively large errors are associated with later responses (36–72 hours), it is important to note that each curve was obtained as the average of four to 16 studies and that there is little random error apparent within each time course profile. The differences can therefore be due only to a systematic effect associated with the different doses. It should also be noted that earlier responses (4–24 hours), with a much smaller error, are statistically different at the extremes of doses employed (16).

Three-Compartment Model

In order to examine the apparent saturation effect further, we have fitted the data to the three-compartment model developed by Reich et al (18) for doxorubicin plasma clearance profiles in man. This model is shown in figure 1, together with the kinetic parameters used in our analysis. The model presented in figure 1 has been simplified slightly from that presented by Reich et al, since the RIA data of Bachur et al (16) were not complicated by an apparent 10% contamination of adriamycinol in TLC fractions of doxorubicin. However, it is well-documented that the plasma levels of doxorubicin, as detected by RIA, contain all of the plasma adriamycinol (23), and this has been taken into account as illustrated in our model (fig 1).

All kinetic parameters used were those evaluated by Reich et al (18), except for L (0,1), the unassigned irreversible loss of doxorubicin from compartment 1, and the delay between the movement of doxorubicin from compartment 1 and its reappearance as adriamycinol in compartment 5. These parameters were allowed to vary in order to optimize the fit (fig 3) between the experimental data and the three-compartment doxorubicin model. The best-fitting values so obtained are presented in table 1, together with the best fitting values for L (0,1) if no delay was permitted. It is apparent from figure 3 and the values presented in table 1 that the data are adequately described by the three-compartment model of Reich et al for all doxorubicin dose levels studied. Table 1 and figure 4 show that the pharmacokinetic parameter L (0,1) clearly takes on a value which depends on the doxorubicin dose used for these patients. This dependence exists irrespective of whether a delay is allowed for between the metabolism of doxorubicin to adriamycinol, and the detection of adriamycinol in plasma, although the agreement between the data and the model is enhanced if a delay does exist. This conclusion is consistent with Reich's model, which also requires a delay between compartments 1 and 5. Furthermore, the 0.5–1.8-hour delays (table 1) are within the range (0.45–8 hours) found by Reich.

Low Dose Level

Since the pharmacokinetic parameters for the 15-mg/m^2 dose deviated from those detected for the higher doxorubicin doses, and we have found that the higher

TABLE 1.—Dose dependence of unassigned loss parameter L (0,1) and adriamycinol synthesis delay*

	Delay optimized			Zero delay	
Dose (mg/m^2)	L (0,1) (hr^{-1})	Delay (hrs)	Sigma	L (0,1)	Sigma
60 + 45	2.01 ± 0.31	1.83 ± 0.77	$0.076.10^{-3}$	1.56 ± 0.20	$0.10.10^{-3}$
30	1.44 ± 0.31	0.86 ± 0.17	$0.15.10^{-3}$	1.16 ± 0.22	$0.17.10^{-3}$
15	1.06 ± 0.13	0.51 ± 0.17	$0.071.10^{-3}$	1.00 ± 0.14	$0.12.10^{-3}$

*Values were calculated at different doxorubicin doses for L (0,1) and the delay, based upon the 3-compartment model shown in fig 1. Errors shown are SD. Sigma = standard error of the fit of the model to the observed data.

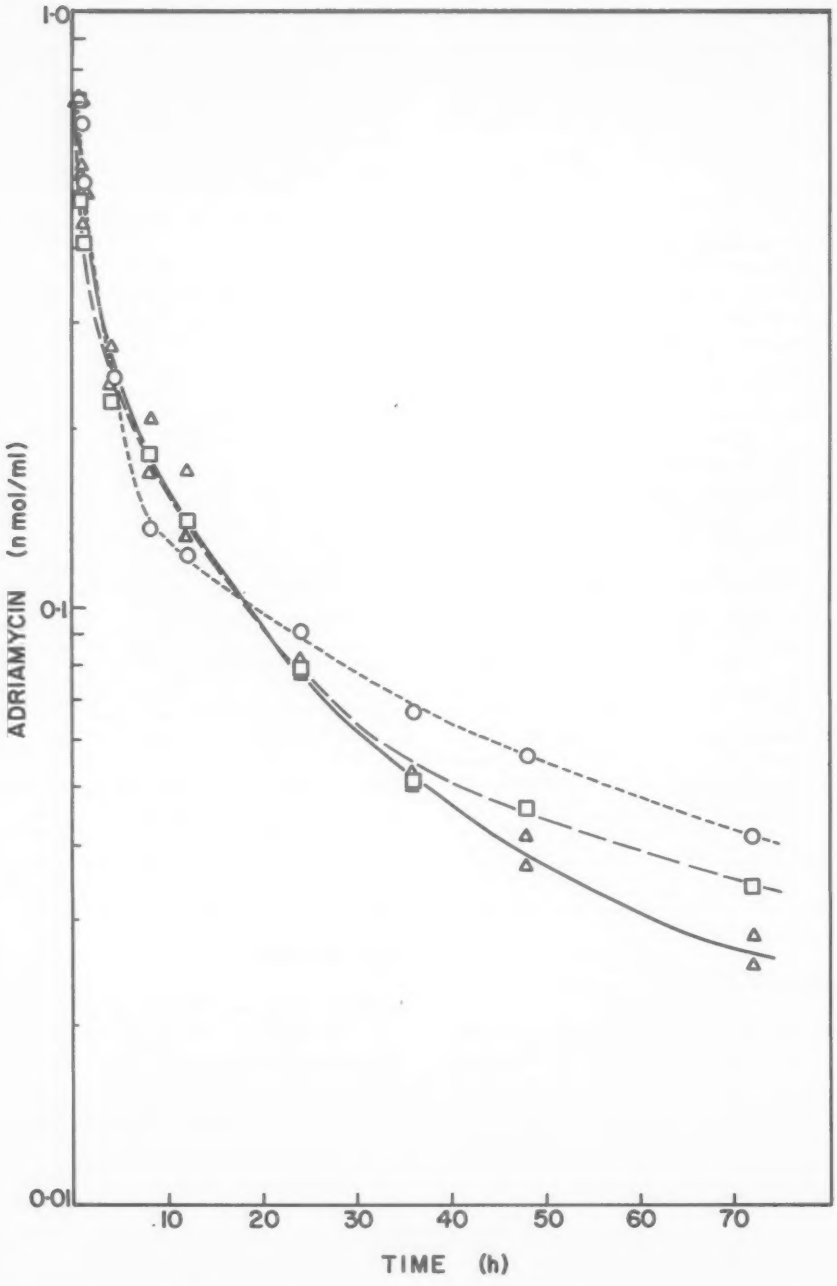

FIGURE 2.—Data of Bachur et al (16) for dose-dependent doxorubicin plasma disappearance in man. Doxorubicin concentrations were determined by RIA. Data for the 45- and 60-mg/m^2 (triangles), 30-mg/m^2 (squares), and 15-mg/m^2 (circles) doses have been juxtaposed by a scaling procedure outlined in the Results section.

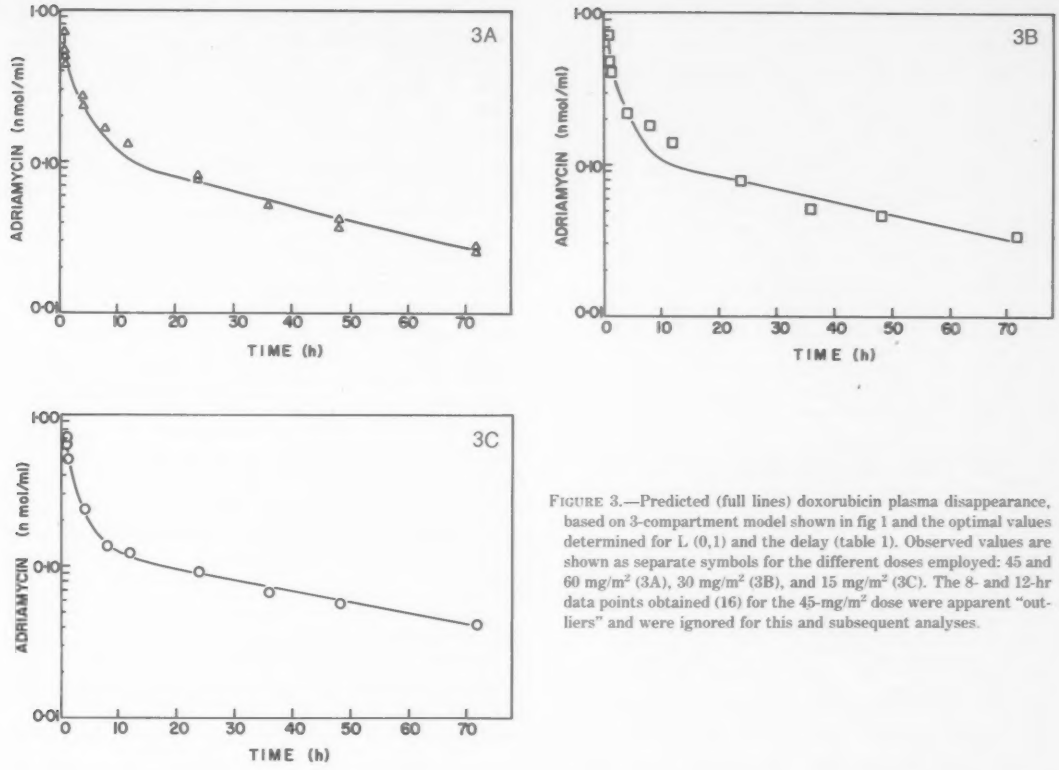

FIGURE 3.—Predicted (full lines) doxorubicin plasma disappearance, based on 3-compartment model shown in fig 1 and the optimal values determined for L (0,1) and the delay (table 1). Observed values are shown as separate symbols for the different doses employed: 45 and 60 mg/m^2 (3A), 30 mg/m^2 (3B), and 15 mg/m^2 (3C). The 8- and 12-hr data points obtained (16) for the 45-mg/m^2 dose were apparent "outliers" and were ignored for this and subsequent analyses.

dose levels can be described by a three-compartment model, it was important to see if the data for the low dose level could be described by a simpler model. Figure 5 shows that the 15-mg/m^2 RIA data of Bachur et al (16) can in fact be adequately described by a two-compartment model. The parameters employed in this model are presented in table 2.

TABLE 2.—Kinetic parameters for 2-compartment model*

Parameter	Value ± SD (hr^{-1})
L (2,1)	0.296 ± 0.043
L (1,2)	0.092 ± 0.012
L (0,1)	0.0879 ± 0.0070

*Values were calculated for a 2-compartment model of doxorubicin disappearance from plasma, based on the experimental data of Bachur et al (16) following iv infusion of a 15-mg/m^2 drug dose.

DISCUSSION

Several lines of evidence suggest that doxorubicin pharmacokinetics may change between treatment levels and that under certain conditions this change may be symptomatic of nonlinear kinetics. The kinetic profiles presented in figure 2 are obviously different. A saturation (thresholding) effect is apparent, since the kinetic profiles change progressively with increasing concentration and become independent of concentration only at a dose level > 45 mg/m^2. Furthermore, the scaling factors required to present the data of Bachur et al (16) juxtaposed in figure 2 were also concentration-dependent, even though the RIA data were normalized relative to the initial dose administered. If the response was linear, data normalization would automatically lead to approximate juxtaposition (apart from experimental error) of the clearance profiles. It should also be noted that the RIA plasma data can be adequately described by a three-compartment model (fig 3) for all four drug dose levels employed. However, the data for the 15-mg/m^2 dose level could be described virtually as well by a simple two-compartment model, whereas the data for the higher dose levels could not be so described. This situation was the same irrespective of whether the adriamycinol contribution to the detected RIA levels was accommodated in the model or ignored.

Since these differences cannot be attributed to experimental error, it must be concluded that there is

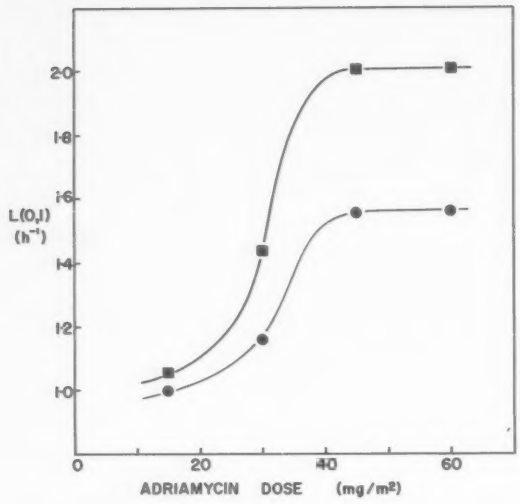

FIGURE 4.—Dependence of the predicted value of L (0,1), loss of doxorubicin from compartment 1 (total body water), upon doxorubicin dose. Model shown in fig 1 was used, setting the delay to zero (circles) or allowing the delay to be optimized (squares).

indeed a dose dependence of doxorubicin kinetics for these patients. However, because the liver status of the patients in these studies was not identical, we stress that it is not possible to identify from these data the source of variation of kinetics with treatment level as being due to either patient type or treatment level. To resolve this ambiguity and to gain insight into the nature of the dependence of doxorubicin kinetics on dose level and patient type, doxorubicin plasma clearance profiles [as well as profiles for the numerous metabolites that have been resolved, particularly using reverse-phase phenyl high-pressure liquid chromatography columns (9)] should be obtained at different dose levels for each patient type. These results are not currently available and, until such definitive studies have been completed, doxorubicin pharmacokinetic data interpreted using linear kinetics should be treated with caution.

The detection of decreasing losses of doxorubicin from plasma with decreasing dose (fig 4) is consistent with prolonged doxorubicin levels at low drug levels (fig 2). This is not unexpected, since the different dose levels were selected on the basis of impaired liver function. These delayed clearance profiles have also previously been observed by Benjamin et al (22) and Chan et al (17) in patients with hepatic dysfunction. It is significant that this effect has also been observed by Piazza et al (19) in patients with normal hepatic and renal function. They measured the intermediate half-life of the triphasic doxorubicin decay in plasma as 252 minutes (20–30 mg/m²), 178 minutes (35–46 mg/m²), and 147 minutes (48–60 mg/m²). Although the experimental errors in this particular study were very large, the general observation that these kinetic profiles exhibited some form of dose dependence suggests that the differences shown in figure 2 may be a real effect of differing doses and not merely an artificial correlation related to the liver status of patients studied by Bachur et al (16).

Chan et al (17) have also obtained pharmacokinetic data from 23 patients given varying doxorubicin doses (10–60 mg/m²). They attempted to fit a ten-compartment physiologic pharmacokinetic model scaled from rabbit tissue distribution data to plasma doxorubicin levels. The time course for most of the patients studied was very similar for the first 4 hours, and this is consistent with the apparent insensitivity of plasma doxorubicin levels to dose shown in figure 2 for the same time interval. Chan et al did not attempt any further examination of any possible dose dependence because of the variation of the plasma tissue course in individual patients. Piazza et al (19) have also reported this problem.

The observation that the low-dose data can be described by a two-compartment model, whereas a three-compartment model is required for higher doses, may explain why there is no consensus as to the model required for doxorubicin pharmacokinetics in man. This suggestion must be treated with caution because of the nature of the patients in the original study by Bachur et al (16). A complete model must account not only for the different metabolites present and different doses, but also for differing degrees of hepatic and renal dysfunction.

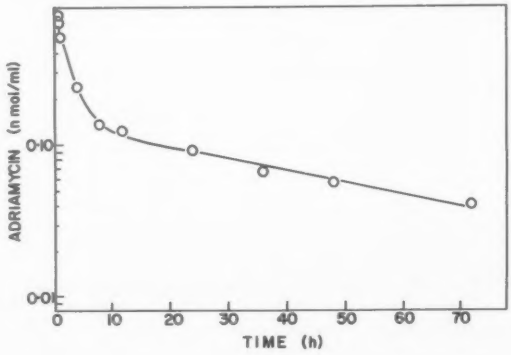

FIGURE 5.—Observed (circles) and predicted (full lines) doxorubicin plasma disappearance, based on a 2-compartment model. Kinetic parameters for this model are shown in table 2.

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Practical Side of Multistage Clinical Trials for Screening New Agents[1,2]

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Coordinating center operations are recommended for carrying out multistage phase II clinical trials in oncology. The relevant statistical issues in designing phase II trials are summarized. The predictive probability approach to multistage design is briefly described, along with an interactive computer program that facilitates design selection. A multistage trial monitoring form is described, along with data manager-biostatistician responsibilities for monitoring the trial for possible early termination. Procedures are recommended for dealing with patients who cannot be evaluated by response, for minimizing or eliminating selection bias introduced by clinician knowledge of interim results, and for minimizing clinician tendency to report responding patients sooner than those not responding. [Cancer Treat Rep 67:71–75, 1983]

Several multistage designs for screening new drugs have been proposed (1–6), but little has been written on the mechanics of carrying out such studies in either a single institution or in a multi-institution cooperative setting. Several authors have written about the practical difficulties inherent in executing sequential clinical trials (7–9). The chief problems mentioned are the administrative burdens of maintaining a constant vigil over the accumulating data and the time lag that frequently exists between patient entry and response determination and between response determination and the reporting of results to the coordinating center. In this paper, coordinating center operations are recommended for designing and carrying out multistage phase II clinical trials in oncology. The first section provides a description of the role and setting of the phase II clinical trials; the second section summarizes the key statistical issues involved; and the third section deals with the practicalities of executing a multistage trial, including the use of an interactive computer program to select the appropriate multistage design, data management procedures for monitoring the trial, and administrative procedures for dealing with potential sources of bias.

PHASE II CLINICAL TRIAL IN ONCOLOGY

Research with a new chemotherapeutic agent in oncology generally progresses through several levels as described by Williams and Carter (10). First, preclinical or animal studies establish the new agent's mode of action and acute toxicity levels. Studies of its action in man begin with the phase I trial which establishes dosage. The recommended dose is then used in the phase II study to determine whether the new agent is of sufficient efficacy to be worthy of further study. The phase III trial is a comparative trial, usually incorporating the new agent in combination chemotherapy regimens.

In most settings, patients first diagnosed as having a particular malignancy are treated in the current phase III trial for that malignancy. Patients relapsing in a phase III trial are then offered treatment in a phase II trial. However, for some cancers, such as colorectal carcinoma or non-small cell carcinoma of the lung, it may be appropriate to place previously untreated patients in new agent trials, because of the lack of effective phase III-type regimens.

STATISTICAL INFERENCE

At some fixed time after the start of therapy, the principal investigator will classify each patient as a responder or nonresponder to therapy, using previously agreed upon objective criteria. In acute leukemia trials, response evaluation generally takes place 28 days after the start of treatment, compared to 42–56 days in solid tumor trials. The measure of treatment effectiveness

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is the response proportion, θ, the proportion of all patients who have responded to therapy.

In a phase II trial, the fundamental question of whether the new agent has sufficient efficacy to be worthy of further study may be formulated in terms of a statistical test of hypothesis. Let

$H_0: \theta = \theta_0$ (null hypothesis)

$H_1: \theta < \theta_0$ (alternate hypothesis),

where θ_0 is the minimally acceptable response proportion to justify further study.

In testing this hypothesis, two types of error can be made: false-positives (recommending an ineffective agent for further study) and false-negatives (declaring an effective agent ineffective). Control over false-positive and false-negative errors is important. A false-negative error may have the effect of discouraging further experimentation with an effective treatment, while a false-positive error may result in future patients being treated with an ineffective treatment. In the formulation of the hypothesis test above, statisticians would use the terms type I error for false-negative and type II error for false-positive results.

Relevant criteria for determination of sample size N would certainly include precision for estimation of θ, levels of type I and type II error, and the expected duration of study. For purposes of illustration, it will be assumed that investigators have found a suitable sample size N and a test of hypothesis that rejects $H_0: \theta = \theta_0$ in favor of $H_1: \theta < \theta_0$ whenever $\leq C$ responses are observed in N patients.

In recent years, investigators have become aware of the need for early termination of phase II trials for ethical reasons when early data indicate that a treatment is ineffective and for early reporting of results when early data indicate that a treatment is effective. Multistage designs have been developed (1–6) where, although a "full" sample size N is declared, patients are entered in k stages of sample size $n_1, n_2 \ldots, n_k$ where $n_1 + n_2 + \ldots + n_k = N$. After each stage is accrued, a decision is made either to terminate the trial or to continue to the next stage on the basis of the number of responding patients observed up to that point. The methods used allow for the control of overall type I and type II errors. Abrupt termination of a single-stage trial on an ad hoc basis does not offer this type of error control. I have recently reviewed the state of the art of multistage phase II clinical trials in oncology (11).

I have previously developed predictive probability phase II designs (4). This method considers multistage designs that have, for every stage, a minimum prespecified "predictive" probability of rejecting the null hypothesis if patient accrual continues to the full sample size. This predictive probability is computed taking into consideration the response rate for patients already accrued. These designs allow for early termination only when early data support the hypothesis of treatment ineffectiveness. Prior beliefs of the relative likelihood of different values of θ may be incorporated using prior probability distributions. When prior information does not exist or cannot be quantified, a noninformative prior distribution, such as the uniform distribution, may be specified. Table 1 presents a predictive probability three-stage design used to test the hypothesis $H_0: \theta = 0.30$ versus the alternative $H_1: \theta < 0.30$. If this design is followed, the probability of making a type I error (rejecting an effective agent) is 0.068, while the power or the probability of not declaring an ineffective agent effective (when the ineffective agent has a response rate of 0.10) is 0.796. When the response rate is as poor as 0.10, the average number of patients needed to make a decision (average sample size) is 17.5. Thus, when an ineffective agent is screened, this scheme is expected to use, on the average, eight fewer patients than when an effective agent is screened.

TABLE 1.—3-stage predictive probability design ($H_0: \theta = 0.30$ vs $H_1: \theta < 0.30$): uniform prior distribution (minimum predictive probability for early termination = 0.85)*

Stage	Sample size	Cumulative sample size	Cumulative critical value	Action
1	9	9	0	Terminate and reject H_0 if no responders observed among 9 patients
2	8	17	1	Terminate and reject H_0 if 1 responder observed among 17 patients
3	8	25	3	Reject H_0 if ≤ 3 responders observed among 25 patients

*Probability of a type I (false-negative) error: 0.068 (significance level). Probability of not making a type II (false-positive) error when $\theta = 0.10$: 0.796 (power). Average sample size when $\theta = 0.10$: 17.5.

CONDUCTING THE TRIAL

Selecting the Design

A computer program known as KSTAGE is available from the author. After an "interactive" question and answer session in which the investigator enters sample size and type I and type II error requirements via a computer terminal, the program prints out several alternative multistage designs meeting these specifications (12).

Monitoring the Trial

The employment of the sequential decision rule requires close patient monitoring. It is necessary to recognize when decision boundaries have been reached so appropriate action may be taken. The time lag between patient registration and patient evaluation for response creates the necessity for special monitoring techniques.

It is assumed that the trial is being conducted in a multi-institutional setting. Investigators at the participating institutions send patient data to a central coordinating center where a biostatistician and data manager are assigned to the trial. A principal investigator, located at one of the participating institutions, is responsible for overall trial supervision.

Logistics of the trial.—Figure 1 illustrates the order in which key events generally take place in the evaluation of the patient, subsequent reporting to the coordinating center, and decision making. The example given is for an acute leukemia trial where response evaluation generally takes place on Day 28. Response evaluation is done later if therapy is delayed due to toxicity. The activities in figure 1 illustrate the close communication that takes place between the data manager and the investigator regarding the status of each patient.

Record keeping.—The multistage phase II trial monitoring form shown in figure 2 is useful for monitoring the progress of trials employing this design. Evaluated patients are entered onto the form chronologically by the date the flow sheet containing the evaluation was received. Ties are broken by date of evaluation. Each stage of the trial constitutes a block on the form. At the end of each block the data manager and biostatistician initial the comparison of cumulative number of responders and critical value. The monitoring forms for all ongoing multistage trials are stored in a loose-leaf notebook, thus facilitating instant access to the current status of all multistage trials.

Dealing With Sources of Bias

Administrative procedures must be designed to reduce potential sources of bias peculiar to sequential designs. The effect of the following sources of bias would be to interfere with the randomness of the sequential response data and thereby increase the number of early responders in the trial, thus increasing the likelihood that a trial for an ineffective agent will proceed to the full sample size. I have previously discussed some of these sources of bias (13).

Patients who cannot be evaluated for response.—Occasionally, patients entered in a phase II trial subsequently cannot be evaluated for response because they died shortly after start of treatment, refused further treatment early in the trial, had inadequate trials due to severe toxic effects, or left the study due to major protocol violations. To avoid selective reporting biases, each of these patients must be accounted for in the final report. There is general agreement on a conservative position of classifying patients of this type as nonresponders (14,15). Investigators are instructed to report early deaths and early withdrawals as soon as they occur. These nonresponders are entered on the monitoring form. Any patient for whom an evaluation is not received within 48 days of the treatment start date is entered as a nonresponder on that date. With careful

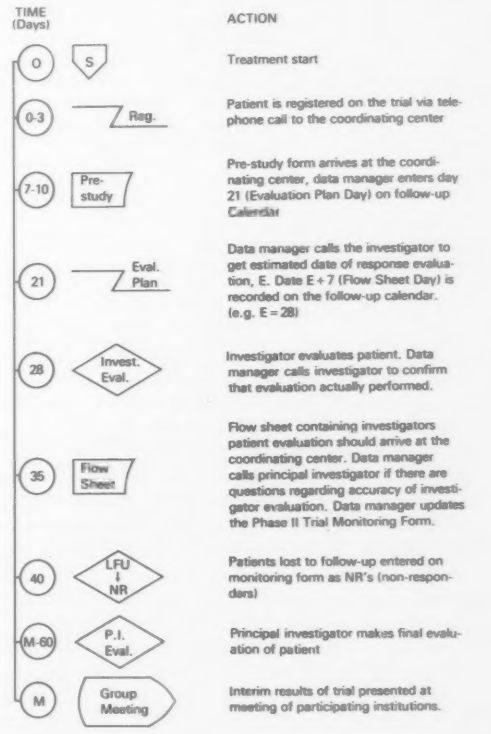
TIME (Days)
ACTION
0
S
Treatment start
0-3
Reg.
Patient is registered on the trial via telephone call to the coordinating center
7-10
Pre-study
Pre-study form arrives at the coordinating center, data manager enters day 21 (Evaluation Plan Day) on follow-up Calendar
21
Eval. Plan
Data manager calls the investigator to get estimated date of response evaluation, E. Date E + 7 (Flow Sheet Day) is recorded on the follow-up calendar. (e.g. E = 28)
28
Invest. Eval.
Investigator evaluates patient. Data manager calls investigator to confirm that evaluation actually performed.
35
Flow Sheet
Flow sheet containing investigators patient evaluation should arrive at the coordinating center. Data manager calls principal investigator if there are questions regarding accuracy of investigator evaluation. Data manager updates the Phase II Trial Monitoring Form.
40
LFU ↓ NR
Patients lost to follow-up entered on monitoring form as NR's (non-responders)
M-60
P.I. Eval.
Principal investigator makes final evaluation of patient
M
Group Meeting
Interim results of trial presented at meeting of participating institutions.

FIGURE 1.—Flowchart describing the monitoring of a multistage phase II clinical trial.

Multistage Phase II Trial Monitoring Form

Study No. 610 Title PLEX--Acute Leukemia

Principal Investigator T. Pride, M.D. Phone No. 212 555-5745

Biostatistician J. Herson, Ph.D Data Manager B. Griffith

Stage I (n_1=9)

	Pt. No.	Invest.	Date	Response
1	20145	Pride	1/20/79	0
2	19021	Cohen	1/27/79	0
3	22802	Cohen	1/30/79	0
4	19990	Mc Dermott	2/1/79	0
5	18005	House	2/20/79	1
6	18222	White	3/9/79	0
7	17719	Mc Dermott	3/12/79	0
8	21141	Hopp	3/26/79	0
9	22611	Pride	4/4/79	0

Total 1
Critical Value 0
Initials GH BH

Stage II (n_2=8)

	Pt. No.	Invest.	Date	Response
10	20086	Oldfield	4/17/79	0
11	21150	Pride	5/2/79	0
12	19567	Hopp	5/20/79	1
13	21166	Schumacher	6/12/79	0
14				
15				
16				
17				

Total
Critical Value 1
Initials

Stage III (n_3=8)

	Pt. No.	Invest.	Date	Response
18				
19				
20				
21				
22				
23				
24				
25				

Total
Critical Value 3
Initials

Stage IV (n_4=)

Pt. No.	Invest.	Date	Response

Total
Critical Value
Initials

FIGURE 2.—Multistage phase II trial monitoring form.

monitoring, it is unlikely that a patient classified as a nonresponder because of loss to follow-up will turn up later as a responder. If this should happen, its consequence would be a premature termination of a trial for an agent that is of borderline efficacy at best.

Selection bias.—If clinicians know the interim results of a phase II trial, this knowledge might cause them, consciously or subconsciously, to select only certain types of patients for future enrollment. One approach to this problem is to "blind" clinicians to interim results. This approach does not allow for collective decision making regarding the course of the trial or in the planning of future trials. Another approach is to have an agreed-upon priority list for each type of malignancy indicating the order in which treatments are offered to patients. Strict adherence to this priority list is enforced. Many investigators feel that blinding interim results is more effective and less time-consuming than trying to enforce a priority list in the absence of blinding. They believe that collective decision making is more important in phase III trials than in phase II trials.

"Wait and see" bias.—Phase II trials are generally nonrandomized. Thus, the clinician is not forced to contact the coordinating center prior to the start of therapy to find out what treatment to apply. A "wait and see" bias can be introduced whenever clinicians can observe initial patient response before deciding whether to register the patient. The requirement that each patient be registered with the coordinating center before treatment begins (or in the event of treatment beginning when the coordinating center is closed, within 3 days of treatment start) is designed to eliminate this source of bias.

"Good news travels first."—Some biostatisticians have conjectured the "good news travels first" theory, whereby an investigator might be likely to report a response to the coordinating center sooner than a nonresponse. A survey of medical records and correspond-

ing flow sheets in clinical trials in which I have participated has yielded no evidence that the "good news travels first" phenomenon occurs in practice. It is important to establish rules for timely reporting. Each patient's flow sheet should be sent within 3 days of the investigator's diagnosis. Telephone contact between the data manager and investigator at the 21- and 29–30-day point is advisable.

Patient heterogeneity.—With good priority list administration, patients entered in phase II trials will generally have had similar prior treatment and be at a similar point in their disease history. Where factors such as presence or absence of distant metastases are known to affect the likelihood of response, stratification on such factors is recommended. A separate phase II trial would be conducted within each stratum. Careful monitoring of patient progress and the reporting process will increase the likelihood of randomness in the patient sequence and thus minimize the chance of making an erroneous conclusion due to early data dominated by an unusual patient group.

Other Motivations for Early Termination

The multistage designs discussed above use patient response as a criterion for early termination. It is understood that other circumstances beyond the control of investigators may also lead to termination. Examples include poor accrual, the presence of other new agents that have promise of greater effectiveness, and unacceptable toxicity.

CONCLUSIONS

This paper has presented coordinating center operations for designing and carrying out multistage predictive probability phase II clinical trials in oncology. The logistics of the patient evaluation and decision-making process in a multi-institution trial were described, along with a record-keeping system for monitoring the trials to assure early termination when warranted. Special attention was given to procedures for conducting the trial in such a way as to minimize nonrandomness in the patient sequence.

Experience shows that a phase II program with 50 patient entries/year will require 20% data manager time for monitoring. The purpose of the monitoring process is to prevent a trial for an ineffective therapy from treating more patients than are specified by the design. Since early responders usually mean that the trial will progress to further stages, the monitoring process is relaxed in these cases, with the priority in monitoring time being placed in those trials for which early-stage termination is still possible.

The data manager is obviously a central figure in the multistage phase II trial process. There is no substitute for a well-trained, dedicated, and self-motivated individual.

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Management of Patients With Metastatic Adenocarcinoma of Unknown Origin: A Southwest Oncology Group Study[1,2]

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The Southwest Oncology Group evaluated 51 patients with a histologic diagnosis of metastatic adenocarcinoma and an occult primary tumor. Even though all patients received an extensive diagnostic evaluation, only six (12%) primary sites were found. Thirty-six patients whose primary tumor sites remained occult were randomized to receive either 5-FU or combination chemotherapy (5-FU, doxorubicin, and cyclophosphamide [FAC]). There were no responses in either group. The median survival with 5-FU was 105 days and with FAC was 95 days. Toxicity with FAC was more common and more severe. [Cancer Treat Rep 67:77–79, 1983]

It is not unusual for a patient to present with metastatic carcinoma and for the site of the primary tumor to remain occult. The incidence has been reported to be between 0.5% and 6.7% of the cancer population (1,2). These patients are diagnostic as well as therapeutic challenges. The overwhelming temptation for the physician is to order a multitude of tests to locate the primary site. The literature now substantiates that this type of approach is unnecessary, most of the time unfruitful, and many times misleading (2–4). Therapeutic management of these patients is uniformly difficult. Treatment with single drugs and combination chemotherapy has yielded a response rate usually $< 20\%$ and a median survival of 2–4 months (5,6).

In 1977, the Southwest Oncology Group initiated a study to: (*a*) determine the yield of specific diagnostic procedures in locating the primary tumor in patients with metastatic adenocarcinoma of unknown primary site (ACUPS), and (*b*) further define responses of ACUPS to chemotherapy. All patients underwent an exhaustive work-up in search of the primary site. Those patients who eventually were given the diagnosis of ACUPS were randomized to receive either single-agent chemotherapy with 5-FU or combination chemotherapy consisting of 5-FU, doxorubicin, and cyclophosphamide (FAC). The findings of that study will be discussed in this report.

MATERIALS AND METHODS

Fifty-one patients from 18 institutions were evaluated in this clinical study. Patients who had a histologic diagnosis of adenocarcinoma which presented at a metastatic site were eligible for study. In addition to a thorough history and physical examination, the work-up included a chest X-ray, three urinalyses, six stools for occult blood, an abdominal computerized axial tomographic scan or echosonography, mammogram (women only), and acid phosphatase (men only). If these studies were negative, bronchoscopy, barium enema, upper GI series, proctoscopy, and gallium and thyroid scans were performed. Bone, liver, and brain scans were done to determine extent of disease at physicians' discretion. If a primary site was found, the patient was removed from the study and treated appropriately. If no primary site was found, the patient was given the diagnosis of ACUPS and then randomized to receive 5-FU or FAC. No patient had had prior chemotherapy. Patients had to have completed one cycle of chemotherapy (28 days) to be evaluable. Clinical characteristics of patients who

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were evaluable for response to chemotherapy are shown in table 1.

FAC doses were 1000 mg/m²/day × 4 days as a continuous infusion (5-FU), 40 mg/m² on Day 1 (doxorubicin), and 400 mg/m² on Day 1 (cyclophosphamide). The 5-FU dose was 1000 mg/m² as a continuous infusion for 4 days. Both regimens were given every 28 days. Once the patient achieved partial remission, the 5-FU infusion could be changed to 500 mg/m² iv weekly at the discretion of the investigator. Poor-risk patients (defined as > 65 years of age, extensive bone marrow involvement, neutropenia < 4000 cells/μl, or thrombocytopenia < 100,000 cells/μl) were given 50% doses of FAC and 60% doses of 5-FU. If grade 1 toxicity was not encountered, 25% dose escalations were permitted. Definitions of response are as follows: *complete remission*—the disappearance of all tumor for a minimum of 4 weeks; *partial remission*—a ≥ 50% decrease in the sum of the products of all diameters of measurable lesion with no new or enlarging lesion for a minimum of 4 weeks; *stable disease*—a < 50% increase or decrease in the size of lesions for at least 8 weeks; and *progressive disease*—a > 50% increase in any lesion or the appearance of new lesions.

TABLE 1.—Clinical characteristics of 36 patients evaluable for chemotherapy

	Treatment	
	5-FU	FAC
Total No. of patients	20	16
Mean age in yrs	59	57
No. of patients with—		
Performance status		
0–2	12	13
3–4	2	1
Unknown	6	2
Poor risk	5	2
Stable disease	7	5
Median survival in days	105	95

TABLE 2.—Sites of presentation

Site	No. of sites*
Liver	22
Lung	16
Bone	15
Nodes	10
Effusions	4
Pleura	2
Skin, brain, bladder	3 each
Eye, retroperitoneum	1 each

*Total = 51 because many patients had multiple sites of involvement at diagnosis.

RESULTS

Of the 51 patients who presented with metastatic adenocarcinoma, 24 presented with an isolated site; liver was the most frequent (13 patients). Other sites of presentation are shown in table 2. Only six (12%) patients had the primary site found after extensive evaluation: two, pancreas; and one each, lung, prostate, gastrointestinal, and colon. Only one primary site was found during the chemotherapy period. This patient had a primary gallbladder tumor discovered at laparotomy. Autopsies were performed in 13 patients. Primary sites were found in 11 patients: pancreas (four), lung (three), kidney (two), and testicular and liver (one each). In two patients the primary site remained occult even after autopsy. In retrospect, seven of 11 patients whose primary site was found at autopsy initially had definitive studies such as an iv pyelogram, abdominal echosonograph, abdominal computerized axial tomographic scan, or bronchoscopy, which should have demonstrated the primary tumor. In these seven patients the studies did not demonstrate the tumor and therefore are classified false-negatives.

Of the 45 patients whose primary tumor was not found, nine were unevaluable for chemotherapy (two refused therapy, three were lost to follow-up, and four died in < 28 days). A total of 36 patients were evaluable for response.

Of the 16 fully evaluable patients who received FAC chemotherapy, there were no observed responses. Eleven of the patients demonstrated progressive disease and five had stable disease. The length of stable disease ranged from 86 to 206 days, with a median of 150 days. The range of survival was 40–736 days, with a median of 95 days. Patients with stable disease survived a median of 275 days and those with progressive disease, 72 days. The patient with the longest survival (736 days) had a gallbladder primary site and after eight cycles of FAC chemotherapy was switched to 5-FU and mitomycin.

Of the 20 fully evaluable patients who received 5-FU as a single agent, no responses were seen. Thirteen patients showed progressive disease and seven had stable disease. The length of stable disease ranged from 53 to 145 days, with a median of 85 days. The range of survival was 28–305 days, with a median of 105 days. Patients with stable disease survived a median of 195 days and those with progressive disease, 81 days.

Toxicity

Four of the 16 (25%) patients who received FAC and 16 of the 20 (80%) patients who received 5-FU had no toxic effects. With FAC therapy there was mild to moderate nausea and vomiting, mucositis, or diarrhea in seven patients, mild to moderate neutropenia (1000–4000 cells/mm³) or thrombocytopenia (50,000–100,000 cells/mm³) in five patients, and severe neutropenia

(< 1000-cells/mm^3) in one patient. With 5-FU there was mild to moderate nausea and vomiting, mucositis, or diarrhea in four patients and mild neutropenia in one patient.

DISCUSSION

The work-up and treatment of patients with ACUPS were addressed in the present study. The fact that this group of patients may comprise close to 7% of an oncologic population makes this diagnosis more common than hypernephroma, ovarian cancer, or pancreatic cancer and deserving of consideration (7).

Approximately 90%–95% of patients who present with cancer will have their primary tumor found with a limited diagnostic work-up. This evaluation should include a history, review of systems, physical examination (including a pelvic and prostate examination), stools for occult blood, urinalysis, chest X-ray, and an acid phosphatase in males. Further radiologic and endoscopic procedures are directed from the results of the initial evaluation (ie, symptomatic areas, positive stools, urine for blood, etc). If this initial evaluation is negative then no further diagnostic studies appear to be necessary.

This limited approach to patients is supported by the results of our study as well as others (3,4). Not only does an extensive evaluation find the primary site infrequently, but it is time-consuming, expensive, uncomfortable, and frequently gives misleading information. In our study, only six of 51 (12%) patients had their primary site found after a very thorough work-up. Nystrom et al (4) reported finding the primary site after an extensive evaluation of 22 of 266 (8%) patients who presented with metastatic adenocarcinomas, and Stewart et al (2) diagnosed eight of 87 (9%). Seven patients in the present study had negative evaluations for kidney, pancreas, and lung primaries, but at autopsy were found to have primary tumors in one of these areas. Nystrom et al (4) reported that of the upper gastrointestinal, barium enema, and iv pyelographic studies done in his study and thought to be diagnostic for the primary site, more than one-half of these were subsequently shown to be false-positives at autopsy. False-negatives, in their review, occurred almost as frequently as true-positives: 14 and 22 cases, respectively.

Patients who present with ACUPS have metastatic disease and in almost all instances are incurable. The diagnostic goal, therefore, should not be to find a primary tumor for which we have limited treatment, but to ensure we do not miss tumors that are curable (lymphoma and germ cell cancer) or tumors that may have frequent responses to hormones or specific combination chemotherapy (prostate, breast, and ovarian). The therapeutic goal for the remaining patients whose primary sites are not found should be to provide palliation and to increase meaningful survival.

To date, chemotherapy for patients with ACUPS has uniformly been unsuccessful. Combination chemotherapy regimens have tried to utilize drugs which have shown activity against the more common cancers which may present as ACUPS (lung, pancreas, kidney, colon, gastrointestinal, and hepatomas). In the present study, FAC appears to be no better than 5-FU alone. FAC did give slightly more durable periods of stable disease, but at the expense of more toxicity. Overall, the median survival of patients was 15 weeks. This is remarkably close to survival data in other studies. Stewart et al (2) reported a median survival of 13–14 weeks in 75 patients treated with various modalities. Woods et al (5), in a randomized study of 47 patients using cyclophosphamide, methotrexate, and 5-FU versus doxorubicin and mitomycin, reported a median survival of 7 and 13 weeks, respectively. Moertel et al (6), in a study of 162 patients, reported a median survival of 4 months for the entire group. Approximately one-half of the patients were treated with 5-FU alone, and the other half were treated with various single agents combined with 5-FU.

As expected, the dismal survival rate of patients with ACUPS parallels the survival statistics of patients with widespread adenocarcinomas of the lung (16 weeks) and pancreas (24 weeks) and hypernephroma (12 weeks). Until there are chemotherapeutic agents that change the prognosis of these tumors, the prognosis of patients with ACUPS will not improve.

Future studies of patients with ACUPS should include a more complete pathologic review with electron microscopy, special stains, immunochemistry, tumor markers, and hormone receptors. The use of these modalities may uncover the primary sites without requiring more diagnostic studies. In addition, utilization of the human tumor cloning assay may provide a better way to select chemotherapeutic agents for this group of patients (8).

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Phase I Evaluation of Oral Tegafur[1,2]

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A phase I clinical study of tegafur (ftorafur) was conducted in 29 patients with various advanced solid tumors. To evaluate its efficacy and toxicity, the initial dose of 0.5 g/m²/day × 21 days at 3-week intervals was progressively increased to a maximum dose of 1.5 g/m²/day. Tegafur was administered orally in two or three divided doses. Diarrhea was the dose-limiting toxic effect and occurred more often in patients with abnormal pretreatment liver function. Nausea occurred in about one-half of the patients, but vomiting was infrequent. Skin rash and mucositis occurred in 10% and 7% of the patients, respectively. Neurologic toxic effects of tegafur were infrequent and mild. The hematologic toxicity of tegafur was minimal. Antitumor activity could be evaluated in 21 patients with measurable disease. One complete and three partial responses were observed in four of 17 patients who had adenocarcinoma of unknown primary origin. All responses occurred at doses ≥ 1.0 g/m²/day. The recommended dose of tegafur for this schedule of administration is 1.0 g/m²/day. [Cancer Treat Rep 67:81–84, 1983]

Tegafur (Mead Johnson) is an antimetabolite closely related to 5-FU, chemically resembling 5-fluorodeoxyuridine (5-FUDR) except that in tegafur the sugar moiety, deoxyribose, is replaced by tetrahydrofuran (1–3). The spectrum of antitumor activity of tegafur in animal tumor systems is similar to that of 5-FU (1,3). Clinical studies with tegafur administered iv demonstrated an antitumor efficacy comparable to that of 5-FU against a variety of adenocarcinomas, including those arising from the breast and the gastrointestinal tract (1,3). Pharmacologic studies suggested that the compound is metabolized slowly to 5-FU in vivo (2,4,5). However, unlike 5-FU, when tegafur was administered by the 5-day iv schedule it was not associated with myelosuppression. The gastrointestinal and neurologic toxic effects were dose-limiting (6,7). Recently, pharmacologic studies with tegafur administered orally indicated that the drug is reliably absorbed from the gastrointestinal tract and that low-dose, long-term administration eliminated the short-term, dose-limiting toxic effects of iv tegafur (8,9). This study was designed to evaluate the efficacy and toxicity of tegafur administered in low-dose repeated daily courses.

MATERIALS AND METHODS

Adult cancer patients admitted to the Department of Developmental Therapeutics were entered in this study. All patients had histologic proof of metastatic cancer. Patients with cancer of known primary origin (eg, gastrointestinal or bronchogenic) were entered in the study after they were no longer responding to conventional treatment of their tumors. For patients with adenocarcinoma of unknown primary origin, tegafur served as the primary treatment. Requirements for entry in the study included recovery from toxic effects of previous therapy, normal blood cell counts, serum creatinine < 1.5 mg/dl, and bilirubin < 1.5 mg/dl.

The starting dose of tegafur was 0.5 g/m²/day for 21 days every 21 days without interruption of tegafur administration between treatment courses. Subsequently, the dose of tegafur was escalated by 0.25 g/m²/day if therapy was not associated with significant toxic effects. Tegafur was administered in divided daily doses: either two or three doses a day so that the daily dose could be given without breaking the tegafur tablets. In accordance with institutional policies, a signed

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consent was obtained from all patients prior to initiation of chemotherapy.

All toxic effects noticed during the courses of therapy were recorded. Cbcs were performed weekly, while urinalysis and renal and liver function studies were repeated at least once every 3 weeks, ie, prior to each course of therapy. Appropriate radiologic examinations as well as radioisotopic scanning procedures were usually performed prior to treatment and after two courses of treatment. Two tumor measurements were recorded in centimeters: the longest diameter and its perpendicular applied at the widest portion of the tumor. Lung lesions were measured by roentgenograms. A complete response was defined as the disappearance of all evidence of active tumor, with the patient free of symptoms for at least 8 weeks. A partial response was defined as a > 50% decrease in the sum of the products of the largest diameters of the lesions. Stable disease was defined as no further growth of a previously rapidly growing tumor for at least 8 weeks or a response that was less than a partial remission. Progressive disease was defined as an unequivocal increase of at least 50% in the size of any measured lesion or the development of new lesions.

RESULTS

A total of 29 patients received 56 courses of tegafur. There were 16 males and 13 females, with a median age of 56 years (range, 31–73). Twenty-four of the 29 patients, all with adenocarcinoma of unknown primary origin, had received no prior chemotherapy. The remaining five patients, including two with carcinoma of the bile duct and one each with adenocarcinoma of the lung, anaplastic carcinoma of the lung, and pancreatic carcinoma, had disease progression while receiving multidrug regimens not containing 5-FU. The dose levels of tegafur ranged from 0.5 to 1.5 g/m^2/day × 21 days. The courses were repeated every 21 days (table 1). Patients were entered at the initial dose level, and subsequently both the starting dose and the dose of the subsequent courses were escalated because no toxicity was seen. In most patients, initial and subsequent therapy was given at dose levels of 1.0–1.25 g/m^2/day (table 1).

Serial blood cell counts were obtained during 49 courses of therapy. Overall, myelosuppression was minimal, as evidenced by the median lowest granulocyte and platelet counts of 4200/mm^3 and 240,000/mm^3 on Days 18 and 20, respectively. Granulocytopenia of < 1000 cells/mm^3 and thrombocytopenia of < 100,000 cells/mm^3 occurred during 4% and 13% of the treatment courses, respectively. The hematologic toxicity of tegafur is summarized in table 2. Myelosuppression, defined as granulocytopenia of < 1000 cells/mm^3 and/or thrombocytopenia of < 100,000 cells/mm^3, was negligible with doses from 0.5 to 1.00 g/m^2/day. At a dose of 1.25 g/m^2/day, however, myelosuppression was seen in about 20% of the courses. The frequency of myelosuppression was higher in patients with pre-existing hepatic function abnormalities (mainly, increased bilirubin and alkaline phosphatase) (table 3). Myelosuppression was generally brief, with recovery of all peripheral blood elements within 6 days. Cumulative myelosuppression did not occur among patients who received three or more courses of tegafur.

Other toxic effects occurred in 62% of the patients and in 45% of the treatment courses. Nausea, often not associated with vomiting, occurred in about one-half of the patients, while diarrhea, anorexia, skin rash, and mucositis were less frequent and occurred in 21%, 14%, 10%, and 7%, respectively. Neurologic side effects were rare. Confusion and generalized weakness occurred in 7% of the patients, while dizziness occurred in 4%. A summary of the nonhematologic toxic effects of tegafur by dose is shown in table 4. The toxic effects were infrequent at doses of 0.5–1.0 g/m^2/day. At the 1.25-g/m^2/day dose level, toxic effects occurred during 73% of the courses. Episodes of nausea of moderate to severe intensity, accompanied by vomiting during 60% of these episodes, occurred in one-half of the courses. It usually appeared during the third week of administration and became dose-limiting with prolonged uninterrupted administration at this dose level. At the 1.5-g/m^2/day dose level, nausea appeared earlier and was more severe. The severity and frequency of nausea and vomiting were reduced somewhat if the daily dose of tegafur was taken during or immediately after meals rather than on an empty stomach. Diarrhea occurred during < 25% of the courses, mainly during the third week of

TABLE 1.—Phase I dose escalation of tegafur

Dose level (g/m^2/day × 21)	Initial No. of —		Subsequent No. of —	
	Patients	Courses	Patients	Courses
0.5	6	10	—	—
0.75	4	6	2	3
1.0	3	5	3	7
1.25	14	15	4	7
1.5	2	2	1	1

TABLE 2.—Hematologic toxicity of tegafur, by dose

	Dose (g/m^2/day × 21)				
	0.5	0.75	1.0	1.25	1.5
No. of evaluable courses	9	9	10	21	2
Lowest neutrophil count					
Median*	4	6.4	4.2	3.3	7.3
Range*	2.6–9	2.8–19	2.5–9.9	0.2–14	2.2–12.5
Day	15	21	15	16	22
% courses					
< 1000 cells/mm^3	0	0	0	11	0
< 500 cells/mm^3	0	0	0	11	0
Lowest platelet count					
Median*	176	251	275	230	430
Range*	62–287	183–702	161–478	47–534	211–650
Day	20	21	20	19	12
% courses					
< 100,000 cells/mm^3	14	0	0	19	0
< 50,000 cells/mm^3	0	0	0	0	0

*Cells × $10^3/mm^3$.

the treatment cycle and, like nausea and vomiting, it was more severe in patients with abnormal liver functions. The diarrhea consisted of eight to 12 loose, watery bowel movements daily, which usually did not respond to antidiarrheal medications unless the tegafur treatment was interrupted. The neurologic toxic effects were in general mild and occurred during 5%–10% of the courses (table 3).

In 11 patients the dose of tegafur was escalated to the point of dose-limiting toxicity. The median total dose of tegafur in these patients was 67 g (range, 32–450) over a median of 42 days (range, 15–165). Patients with normal liver functions tolerated a larger tegafur dose (median, 75 g; range, 35–450) than patients with abnormal liver function tests (median, 52; range, 32–103). In these patients, the dose-limiting toxic effects were diarrhea (seven patients), mucositis (three), and nausea (one).

Twenty-one patients received treatment with tegafur for ≥ 6 weeks and were evaluable for response. The remaining eight patients included two with no measurable disease, four with inadequate trials, one who returned home and refused further follow-up, and one who died from disease progression before completing 3 weeks of treatment. The remaining 21 evaluable patients included 17 with adenocarcinoma of unknown primary origin, four of whom responded. There was one complete response lasting 6 months in a patient with cervical lymph node metastasis; three partial responses, each lasting 3 months, were observed in patients with lymph node metastasis, subcutaneous mass plus lymph node metastasis, and liver plus lung metastasis. Partial responses occurred at dose levels of 1.0, 1.25, and 1.5 g/m^2/day, while the complete response was observed at a dose level of 1.25 g/m^2/day. Five of 21 evaluable patients had disease stabilization, while 12 patients had disease progression despite treatment with tegafur for up to 6 weeks.

DISCUSSION

Our phase I study of oral tegafur administered daily confirms the potential usefulness of the compound. It is well-tolerated and has antitumor activity comparable to that of 5-FU. The maximum tolerated daily dose was 1.25 g/m^2, and gastrointestinal toxicity was the dose-limiting toxicity. Although nausea and vomiting were the most common manifestations of gastrointestinal toxicity, they were infrequently dose-limiting. However, diarrhea, although infrequent, necessitated discontinuation of tegafur. Neurologic toxicity occurred at a dose level of 1 g/m^2/day. Symptoms consisted of speech difficulty, confusion, and dizziness; unlike the experience with iv tegafur, such toxic effects were infrequent

TABLE 3.—Toxic effects, by liver function

	Liver function tests	
	Normal	Abnormal*
No. of evaluable courses	38	15
% nausea	24	40
% vomiting	11	20
% thrombocytopenia†	3	20
% diarrhea	11	13
% neutropenia‡	0	13
% mucositis	3	7

*SGOT and alkaline phosphatase > 1.5 × normal.
† < 100,000 cells/mm^3.
‡ < 1000 cells/mm^3.

TABLE 4.—Nonhematologic toxic effects, by dose of tegafur

	Dose (g/m²/day × 21)					
	0.5	0.75	1.0	1.25	1.5	Overall
No. of evaluable courses	10	9	12	22	3	56
% courses with toxicity	10	22*	33	73	66	45
% nausea†/vomiting	—	22*	25	50	33	30
% diarrhea	—	—	8	23	33	13
% anorexia	10	—	—	9	33	7
% mucositis	—	—	—	9	—	4
% speech difficulty	—	—	—	9	—	4
% weakness	—	—	—	9	—	4
% skin rash	—	—	8	5	33	5
% dizziness	—	—	—	5	—	2
% confusion	—	11*	—	5	—	4

*Patient had symptomatic congestive heart failure.
†Moderate to severe.

(2,6,7). These central nervous system side effects have been explained by neuropharmacologic studies revealing that tegafur readily crosses the blood-brain barrier (4).

The myelosuppressive effect of tegafur administered orally was minimal and did not differ from that associated with iv tegafur (6,7). This has been explained by the slow degradation of tegafur to 5-FU, simulating slow, constant infusion of 5-FU, which is less myelosuppressive than 5-FU bolus iv administration (10,11). The incidence of gastrointestinal and myelosuppressive toxic effects was closely related to the presence of abnormal hepatic function.

The spectrum of antitumor activity of oral tegafur could be established only by studying a larger number of patients with various types of malignancies. However, the results of the present study suggest that oral tegafur at a dose of 1 g/m²/day for ≥ 21 days could be effective for the treatment of human malignancies. A response rate of 24% in patients with adenocarcinoma of unknown origin suggests an improvement when compared to the 16% response rate observed with 5-FU administered iv to similar patients (12). In view of the minimal myelosuppressive effect associated with tegafur, this compound might be used in combination with myelosuppressive agents without the need for a significant dose reduction.

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Fatal Congestive Heart Failure Following Mitoxantrone Treatment in Two Children Previously Treated With Doxorubicin and Cisplatin[1,2]

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Two children, who had received chemotherapy with doxorubicin and cisplatin for disseminated chondrosarcoma and recurrent rhabdomyosarcoma, developed congestive heart failure following treatment with mitoxantrone (DHAD); the total doses of DHAD were 128 and 90 mg/m², respectively. The patient with chondrosarcoma had relief of his cardiac symptoms with digitalis and furosemide. The patient with rhabdomyosarcoma, whose tumor responded following treatment with DHAD, died 2 weeks after development of congestive heart failure. For patients who have received prior treatment with potentially cardiotoxic agents, administration of DHAD should be followed by careful monitoring of cardiac function. [Cancer Treat Rep 67:85–88, 1983]

Mitoxantrone (DHAD) is an anthraquinone that was developed to provide a quinone-containing antitumor agent devoid of cardiotoxicity by replacement of the amino sugar with an amino alkyl side chain. It was also hoped that this change would provide enhancement of intercalation with the nucleic acid, in a manner similar to that provided by doxorubicin (1–5).

DHAD has antineoplastic activity against a variety of animal tumors and leukemias (6,7). Its toxicity in animal systems included bone marrow, gastrointestinal, and lymphoid system toxicity. In the therapeutic range of animal doses, cardiotoxicity was not observed. However, cardiotoxicity, manifested by reduction in the left ventricular function, diastolic pressure, and cardiac output, occurred when higher doses were administered to beagle dogs (8).

Cardiotoxicity was not observed in initial trials involving adults (9–14). A recent report by Schell et al (15) indicates that four patients with breast cancer previously treated with doxorubicin developed congestive heart failure (CHF) during or after treatment with DHAD. We now report two pediatric patients in whom CHF followed treatment with this agent.

CASE REPORTS

Patient 1

Patient 1 was a $7^1/_2$-year-old boy who was admitted in October 1977 for treatment of metastatic extraosseous chondrosarcoma after amputation of the right leg. His initial treatments consisted of weekly high-dose methotrexate (7.5 g/m²) alternated with doxorubicin (75 mg/m²) and cyclophosphamide (600 mg/m²) until a total doxorubicin dose of 525 mg/m² was delivered; his ECG at that time was normal. Following bilateral thoracotomies, new pulmonary metastases were observed. Other sequential chemotherapy included cisplatin and ICRF-187. In July 1980, with increasing size of pulmonary metastases, the patient was admitted to the phase I DHAD protocol of the pediatric subgroup, Phase I Working Group (16). Heart size and ECG were normal. DHAD (18 mg/m²) was delivered monthly, without an increase in the size of lung lesions. Sequential echocardiograms demonstrated progressive decrease in percent shortening of the lateral minor axis of the left ventricle (shortening fraction), with concurrent in-

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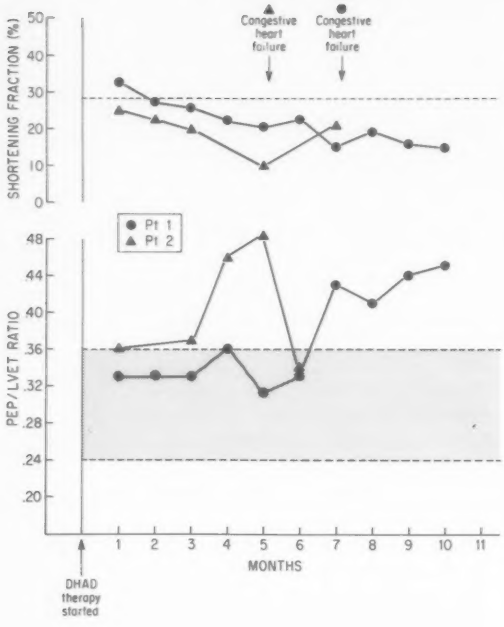

FIGURE 1.—Cardiac status in 2 children who received DHAD.

crease in the pre-ejection period to left ventricular ejection time (PEP/LVET) ratio (fig 1); cardiac blood pool scans were not performed. One month after the seventh dose of DHAD, the patient presented with tachycardia and tachypnea; chest roentgenogram demonstrated cardiomegaly. The M-mode echocardiograms showed further decrease of the shortening fraction to 15% and increase in the PEP/LVET ratio to 0.45 (> 2 SD of normal mean), suggestive of CHF. The patient was given digoxin and furosemide, with improvement over several months prior to death from pulmonary insufficiency. Autopsy was not performed.

Patient 2

Patient 2 was a 4-year-old girl who was admitted in May 1977 for treatment of rhabdomyosarcoma of the left buttock (with spread to the left inguinal lymph nodes). Treatment included vincristine, mechlorethamine, dactinomycin, and doxorubicin over $1^1/_2$ years. ^{60}Co radiation was delivered to the primary tumor and metastases beginning 6 weeks after the start of chemotherapy. Radiation therapy doses were 5028 and 3300 rads, without scatter to the heart. The total dose of doxorubicin delivered before completion of planned therapy was 125 mg/m^2.

Recurrent tumor in the left renal region was found in May 1980. The patient responded to treatment with cisplatin followed by doxorubicin but 4 months later these agents were discontinued because of evidence of renal dysfunction and minimal changes in the echocardiogram. She had received 295 mg/m^2 of doxorubicin during her entire treatment period. During a period of observation while no additional treatment was given, her ventricular shortening fraction decreased from 29% to 20%. After flank recurrence, she was treated with 18 mg/m^2 of DHAD on five occasions. Sequential echocardiograms indicated decreases in the shortening fractions and increases in the PEP/LVET ratio (fig 1). The flank mass again disappeared.

In June 1981, the patient presented with CHF. Echocardiogram showed a shortening fraction of 5% and a PEP/LVET ratio of 0.43. Multigaited cardiac blood pool study (MUGA) demonstrated diffuse hypokinesis of the left ventricular wall and a left ventricular ejection fraction of 0.23. With digitalis and furosemide, initial improvement was followed by acidosis, cyanosis, and death. Autopsy was performed 13 hours after death; the heart weighed 150 g (expected weight for age, 100 g). Microscopically, varying degrees of focal vacuolar degeneration were noted within myocytes (fig 2). In some areas, myocyte nuclei were enlarged, hyperchromatic, and irregular in shape. Hemorrhage, necrosis, and inflammation were not seen in the heart, and tumor was not found in any area.

DISCUSSION

We report two children who developed CHF after receiving DHAD. Both of these patients had received prior chemotherapy including doxorubicin and cisplatin. One patient had also received iv cyclophosphamide. Doxorubicin (17–28) and cyclophosphamide (29–32) are known to produce cardiac dysfunction and cardiomyopathy, usually within 6 months following therapy, although abnormal cardiac changes may present for years. Cisplatin has been associated with arrhythmias (33,34). Cardiotoxicity associated with cyclophosphamide administration has followed treatment with extremely high doses (29–32). Although other agents such as dactinomycin and mithramycin (35) have been demonstrated to exacerbate doxorubicin cardiomyopathy, DHAD was specifically synthesized to have little if any cardiotoxic activity.

The myocardial lesions found in patient 2 are nonspecific but correspond to the early changes described in doxorubicin cardiomyopathy (23). These changes may also be associated with CHF, anemia, hypoxia, and acidosis. For both patients, it is difficult to be certain whether DHAD played a role in the production of these myocardial lesions. In both of our patients, neither of whom had clinical evidence of heart failure prior to

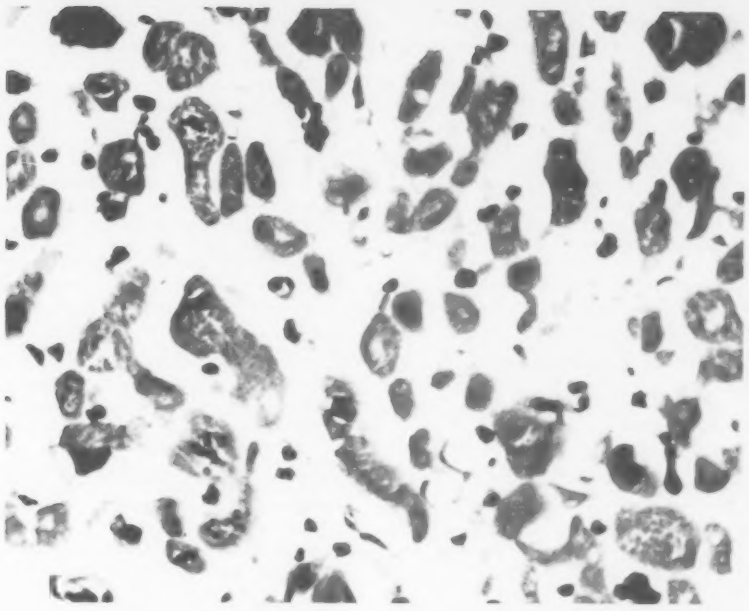

FIGURE 2.—Myocardium demonstrating myocyte vacuolar degeneration and nuclear irregularity (× 240).

treatment with DHAD, this agent may have exacerbated previously unrecognized myocardial dysfunction by producing additional cardiac lesions, or DHAD may not have contributed in any way to the development of CHF.

Patient 2 received only 295 mg/m^2 of doxorubicin and had evidence of prior cardiac dysfunction demonstrated by sequential echocardiograms. The development of CHF by patients receiving DHAD has led to the recommendation that this agent be used in patients who have received no more than a 450-mg/m^2 total dose of doxorubicin (L. Lev, personal communication) and who have had no previous cardiac abnormalities. It is our additional recommendation that patients not receive DHAD unless there has been assurance of normal cardiac function, including normal ECG, echocardiogram with shortening fractions > 28%, and normal heart size by chest radiograph. Further, treatment of patients receiving DHAD should be limited to two courses prior to evaluation of tumor response. If no responses have been obtained following the delivery of two courses on the 3-week schedule, further treatment with DHAD should not be delivered.

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Phase II Trial of Vindesine in Advanced Gastric Cancer[1]

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For the North-Western Gastro-Intestinal Tumour Group

Fifteen consecutive patients with measurable advanced or metastatic gastric adenocarcinoma were entered in a phase II study of vindesine. An iv bolus injection of vindesine (3 mg/m^2) was given weekly × 4 and twice weekly × 2. There were no objective responses among the 14 evaluable patients. [Cancer Treat Rep 67:89–90, 1983]

Vindesine is a semisynthetic vinca alkaloid. Initial studies have suggested a wide range of antitumor activity and toxic effects between vincristine and vinblastine (1). Phase I trials have recommended vindesine at a dose of 3–4 mg/m^2 iv every 1–2 weeks (1). Leukopenia was dose-limiting at higher doses, and peripheral neuropathy prevented more frequent administration. Phase II trials have shown vindesine to be active in a number of different tumors (2).

Gastric cancer is the most responsive of all gastrointestinal adenocarcinomas to chemotherapy, but responses are seldom complete and are usually short-lived. At present, the major active agents are doxorubicin, 5-FU, mitomycin, methotrexate, and the nitrosoureas. The best combinations of these agents produce a 30%–50% response rate (3). New active agents are needed, preferably with nonoverlapping toxic effects. It was proposed therefore to evaluate the activity of vindesine and gastric cancer.

MATERIALS AND METHODS

All patients had histologically proven adenocarcinoma of the stomach or the cardioesophageal region. No patient had received previous chemotherapy or radiotherapy. A normal leukocyte count, platelet count, and a Karnofsky performance status ≥ 40% were criteria for entry in the study. All patients had objective evidence of disease by clinical bidimensional measurement, chest X-ray, or laparoscopy. Laparoscopy is routinely used by one professorial surgical unit to assess operability. If liver metastases were detected, their location, size, and number were noted. Repeat laparoscopy performed by the same experienced surgeon was found to be accurate and reproducible in assessing visible liver metastases. Abdominal ultrasound and computerized tomographic scanning were also acceptable if they showed an unequivocal clearly defined measurable lesion. Isotope scans were not accepted.

An iv bolus injection of vindesine[6] (3 mg/m^2) was given weekly × 4 and then twice weekly × 2.

In the absence of objective disease progression, all patients were reassessed after six injections (8 weeks). All abnormal investigations at presentation were repeated, including laparoscopy, if that was the only objective tumor reassessment available. A partial response was defined as a 50% reduction in the product of the two largest perpendicular diameters.

Patients with stable disease at the time of reassessment and no significant toxicity continued to receive maintenance vindesine every 2 weeks.

STATISTICS

A two-stage design for a phase II trial was used (4). If no responses are observed among the first 14 evaluable patients, the true response rate is almost certainly between 0% and 19% (95% confidence interval) and the trial can be terminated.

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[5]We thank members of the North-Western Gastro-Intestinal Tumour Group for referring patients, and P. Willett for typing this paper.

[6]Supplied by Eli Lilly and Co, Basingstoke, Hants, England.

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TABLE 1.—Patient characteristics

Characteristic	
Males	11
Females	4
Median age in yrs (range)	59 (40–71)
Median Karnofsky performance status (range)	70% (40%–90%)
Median duration of symptoms in mos (range)	4.5 (1–9)
Median time from diagnosis to chemotherapy in mos (range)	1.5 (0.5–4)
No. of patients with—	
Primary inoperable	11
Main site evaluable (main method)	
Lung 2nd (chest X-ray)	2
Cervical nodes (palpable)	2
Abdominal mass (palpable)	4
Liver 2nd (ultrasound)	3
Liver surface 2nd (laparoscopy)	4

RESULTS

Between October 1980 and August 1981, 15 consecutive patients with advanced gastric cancer were entered in the study (table 1).

There were no objective responses among the first 14 evaluable patients. Three patients had disease progression prior to reassessment (two after four injections and one after five). At reassessment, an additional seven patients were found to have progressive disease. All of the patients with progressive disease died within 5 months, except for one patient whose disease progressed very slowly for 8 months, and who remains alive with combination chemotherapy at 10+ months.

Four patients had stable disease. One patient declined maintenance therapy and is alive at 8+ months. The other three patients received vindesine every 2–3 weeks for 7, 8, and 12 months; they lived 7, 11+, and 16 months, respectively.

Toxicity

The drug was generally well-tolerated with (reversible) peripheral neuropathy being dose-limiting. Thirteen of 15 patients had paresthesias, two had mild motor impairment, and one had severe neuropathy with marked weakness, and a foot drop. Constipation requiring laxatives occurred frequently, but was never severe.

Hematologic toxicity was slight, with only minor drops in the wbc count and hemoglobin level. No thrombocytopenia or marked thrombocytosis was seen. Alopecia was milder than generally reported. Fever, malaise, and/or myalgia were seen on a few occasions.

DISCUSSION

The use of a two-stage trial design allowed completion of the study after 14 evaluable patients showed no responses.

The patients studied were younger and had better Karnofsky performance status than usual for this disease. All patients had an adequate trial of treatment. Vindesine can be given at marginally higher doses, but only at the expense of significantly increasing toxicity. Vindesine is not active in gastric cancer at the dose and schedule used in this trial.

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Phase II Trial of Mitoguazone in Patients With Advanced Squamous Cell Carcinoma of the Head and Neck[1]

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We used mitoguazone (500 mg/m² iv weekly, with 50-mg/m² escalations weekly as tolerated) to treat 22 patients with squamous cell carcinoma of the head and neck which recurred after initial therapy. Nine of 22 (41%) patients responded: two had complete responses and seven had partial responses. Gastrointestinal toxicity and anemia were commonly seen. We conclude that mitoguazone is active in squamous cell carcinoma of the head and neck and should be incorporated into phase III trials. [Cancer Treat Rep 67:91–92, 1983]

Squamous cell carcinoma of the head and neck is a chemotherapy-sensitive tumor. Despite a number of active drugs, prognosis is poor for patients with head and neck squamous cell carcinoma who relapse after local treatment; median survival is about 6 months (1). Thus, there is a need for new agents to combine with known effective agents to improve prognosis in this disease. We report the results of a trial using mitoguazone (Methyl-G) in advanced head and neck cancer.

PATIENTS AND METHODS

Twenty-two patients with advanced squamous cell carcinoma of the head and neck were entered in the study. All patients had failed conventional radiation and surgery and 15 had failed prior chemotherapy. Patients were required to have biopsy-proven recurrence, measurable disease, performance status of 0–3 (Eastern Cooperative Oncology Group), wbc count $> 4000/mm^3$, platelet count $> 100{,}000/mm^3$, and serum creatinine ≤ 1.5 mg/dl. At least 4 weeks had to have elapsed from prior radiation or chemotherapy. Pretreatment evaluation included history and physical examination, cbc and platelet count, urinalysis, multiphasic chemistry profile, chest X-ray, ECG, and scans and X-rays as needed for tumor measurement. All patients provided signed, informed consent.

Response criteria.—Complete response was defined as the complete disappearance of all disease. Partial response was defined as a reduction by at least 50% of the product of dimensions of all measurable lesions.

Method of administration.—Methyl-G was reconstituted in 500 ml of 5% dextrose in water or normal saline and infused through a side port of a freely running iv. The drug was infused over 30–60 minutes. In those patients complaining of circumoral paresthesias, the infusion was slowed to 60–90 minutes. The initial dose of Methyl-G was 500 mg/m² iv weekly. The dose was escalated by 50 mg/m² weekly in the absence of toxicity. A minimum of four doses were given in the absence of progressive disease. During the course of the study, six of nine patients receiving > 6 weeks of treatment were converted to an every-other-week administration schedule to reduce toxicity.

RESULTS

Pretreatment characteristics are shown in table 1. Twelve of 15 patients who had received prior chemotherapy had received adjuvant therapy with cisplatin, bleomycin, and vinblastine at the time of initial diagnosis.

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TABLE 1.—Patient characteristics

	No. of patients	No. of responses
Total	22	
Median age in yrs (range): 62 (24–83)		
Median performance status (range): 1 (0–3)		
Primary sites		
Oral cavity	7	4
Oropharynx	6	4
Hypopharynx	1	1
Larynx	7	0
Nasopharynx	1	0
Relapse sites		
Primary site	11	3
Regional nodes	11	5
Lung	7	1
Bone	2	0
Brain	1	0
Liver	1	0
Prior treatment		
Radiation therapy only	8	3
Surgery only	2	0
Radiation and surgery	12	6
No chemotherapy	7	4
Chemotherapy	15	5
Adjuvant	12	5
Relapse	3	0

TABLE 2.—Toxic effects

	No. of patients
Anemia (Hgb, 7–11 g/dl)	11
Platelet count of 120,000 cells/mm^3	1
Nausea, vomiting, diarrhea	15
> 10% weight loss	5
Transaminases	4
Creatinine rise to < 2 mg/dl	2
Paresthesias	2
Rash	1

Therapeutic Effects

The median dose of Methyl-G attained in the study was 550 mg/m^2 (range, 500–900). In patients who received five or more courses of Methyl-G, the median dose was 600 mg/m^2. The median number of doses administered was six (range, one to 26). All 22 patients were considered evaluable for response.

Two patients had a complete response. One patient had resolution of a pulmonary nodule, lasting 107 days; another had complete regression of a mass at the base of the tongue, lasting 60+ days. Seven patients had partial responses lasting a median of 107 days (range, 28+ to 267). The median time to response was 35 days (range, 28–65). Three patients remain in the study at the time of this report. In all, nine of 22 patients responded, for a 41% response rate. With nine responses among 22 patients, there is a 95% chance that the true response rate is > 20% (2). Median survival for responders was 230 days; for nonresponders, 76 days. This difference was not significant [$P = 0.18$, log-rank (3)].

Toxicity

Toxic effects are shown in table 2. Because of gastrointestinal toxicity, six of nine patients receiving Methyl-G for > 6 weeks required shifting to an every-2-week regimen. This reduced the anorexia and weight loss.

DISCUSSION

In one previously reported trial in head and neck cancer, Methyl-G produced three partial responses among 16 patients, lasting 1, 1$^1/_2$, and 3 months (4). We have found a 41% response rate to Methyl-G among 22 patients with relapsed head and neck squamous cell carcinoma. There are several reasons why this group would be expected to respond (5). First, the median performance status was 1; only two patients had a performance status of 3. Second, only three patients were given Methyl-G immediately after failing another chemotherapy regimen. Seven patients had received no prior chemotherapy; 12 of the 15 who had received prior chemotherapy received adjuvant treatment at the time of initial diagnosis > 6 months previously.

In summary, Methyl-G is an active agent in squamous cell carcinoma of the head and neck. It should be tested in phase III trials.

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Phase I–II Evaluation of Acronine in Patients With Multiple Myeloma[1]

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Acronine (1) is an alkaloid prepared from the bark of *Achronychia baueri*. A high order of activity has been noted against X5563 myeloma in experimental animals. In a phase I study some activity was demonstrated against multiple myeloma (2). A phase II study of acronine in multiple myeloma was therefore indicated.

Sixteen patients with multiple myeloma resistant to conventional treatment were treated daily with oral acronine (Eli Lilly & Co, Windlesham, Surrey, England) starting at a daily dose of 200 mg/m^2 for the first month, thereafter increasing monthly by 100 mg/m^2/day, to a maximum tolerated dose (maximum, 500 mg/m^2/day). Patient characteristics are shown in table 1.

All patients complained of anorexia initially; nausea and vomiting was dose-limiting in one-half of the patients. Seven of the 11 patients who received a daily dose of $\geq$ 300 mg/m^2 had episodes of nausea and vomiting. Treatment was stopped in one patient because of intolerable vomiting.

A rise in liver enzyme levels was noted in one patient who received 300 mg/m^2 of acronine daily for 14 weeks. Mild myelosuppression was observed in two patients, but they both had rapidly advancing disease with increasing bone marrow infiltration.

One patient who received acronine for a total of 93 weeks up to a maximum daily dose of 400 mg/m^2 developed severe ataxia. The ataxia gradually disappeared over a period of 8 weeks after acronine treatment was stopped.

Objective response (2) was seen in only one of the 16 patients treated with acronine (table 1). This was the same patient who received acronine for 93 weeks before the drug was stopped because of the development of ataxia. No response was noted until a daily dose of 400 mg/m^2 was reached. There was then a drop in 24-hour Kappa light chain excretion from 3335 to 563 mg. This response was maintained for 72 weeks, during which the patient remained pain-free and active.

Two additional patients showed decreases in paraprotein, but they were not sufficient to record as objective responses. One patient had a drop in Lambda light chain excretion from 1329 to 750 mg/24 hours, which was maintained for 20 weeks. As with the responding patient, little or no drop in light chain excretion was noted before a daily dose of 400 mg/m^2 was

TABLE 1.—Patient characteristics, and relationship of nausea and vomiting and response to daily dose of acronine

	No. of patients
Entered/evaluable	16/16
Male/female	10/6
Previous chemotherapy	16
Age in yrs	
Median: 67	
Range: 47–76	
Stage*	
IIIA	15
IIIB	1
Maximum daily dose†	
200 mg/m^2	5
Nausea and vomiting	1
Response‡	0
300 mg/m^2	8
Nausea and vomiting	5
Response‡	0
400 mg/m^2	3
Nausea and vomiting	2
Response‡	1

*Durie and Salmon staging system (ref 3).

†Median time from diagnosis to start of acronine was 24 mos (range, 11–69); median survival time from start of acronine was 5.5 mos (range, 2–27).

‡Objective response to treatment, defined by the Committee of the Chronic Leukaemia and Myeloma Task Force (1973) as follows: (*a*) $\geq$ 50% reduction in the product of the 2 largest diameters of plasmacytomas, as assessed by palpation or X-ray; (*b*) fall of M protein to $\leq$ 50% of prestudy value; (*c*) if urinary M protein was $>$ 1 g/24 hrs, fall to 50% of this value, or if urinary M protein was 0.5–1 g/24 hrs, fall to $<$ 0.1 g/24 hrs; and (*d*) definite radiographic evidence of skeletal healing.

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achieved. The other patient had a decrease in IgG Kappa monoclonal protein from 73 to 46.5 g/L, which was maintained for 4 weeks at a daily dose of 300 mg/m^2 of acronine. Unfortunately, the drug had to be stopped at that time because of severe nausea and vomiting.

Acronine showed minimal activity in this study, but the dose used was clearly limited by nausea and vomiting. If a more tolerable formulation of the drug became available, further studies would be indicated at higher doses.

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Cancer and Leukemia Group B Phase II Non-Small Cell Lung Carcinoma Trial: Aziridinylbenzoquinone (AZQ)[1,2,3,4]

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Aziridinylbenzoquinone (AZQ) is one of a class of quinones that has exhibited significant activity against ip implanted L1210 lymphoid leukemia (1) and has increased the lifespan of ependymoblastoma-bearing mice (2). The compound is nonpolar and lipophilic. Phase I studies (3) at two institutions established that the dose-limiting toxic effect in man was myelosuppression; nausea, vomiting, diarrhea, and stomatitis were also reported. Responses in melanoma and adenocarcinoma were seen. Hence, trial of this new and potentially active agent in non-small cell lung cancer, a disease against which effectiveness of standard agents alone or in combination is quite variable but generally disappointing (4), was initiated in the hope that AZQ would be found active in this disease.

PATIENTS AND METHODS

Eighty-eight consecutive patients with locally advanced or metastatic lung cancer or a histologic type *other than small cell* were entered in the study. Principal characteristics of the 74 fully evaluable patients are summarized in table 1. *No evaluated patient had received any prior chemotherapy.* All patients except one were ambulatory and of good performance status (0–2). Disease was measurable in 82.4% of the patients and evaluable in only 17.6%. AZQ was administered as an iv infusion at a dose of 30 mg/m^2 in 100 ml of normal saline over 30 minutes every 21 days; the dose was escalated to 35 mg/m^2 if the wbc and platelet counts did not fall below 3500 and 100,000/mm^3, respectively, with Day-21 wbc count $\geq$ 4000/mm^3 and platelet count $\geq$ 100,000/mm^3. Treatment was continued as long as the disease was stable or responding, in the absence of excessive toxic effects. Partial response in patients with measurable disease was defined as a $\geq$ 50% decrease in the product of two diameters of all measurable lesions.

RESULTS

There were no complete responses. Of the four partial responses, the best was in a patient with large cell carcinoma, where a 6.7-cm mass totally regressed for 10 months and treatment was terminated by an anaphylactic response. The probability that the true response rate of AZQ is at least 20% is not supported by these data ($P < 0.0001$).

Toxic effects were mild, particularly nausea and vomiting. Hematologic toxicity was generally mild, although moderately severe and progressive anemia was observed in almost one-third of the patients.

In conclusion, this phase II trial of AZQ in patients with non-small cell carcinoma of the lung appears to have demonstrated very modest evidence of antitumor effect at a dose level that produced definite but mild and acceptable hematologic toxicity. It is doubtful that the quality of the four partial remissions warrants ad-

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[3]AZQ was supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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TABLE 1.—Patient characteristics and treatment results*

Characteristic	No. of patients (%)
Total evaluable	74
Median age: 60.5 yrs	
Performance status	
Not given	3 (4.1)
0	17 (23)
1	40 (54.1)
2	13 (17.6)
3	1 (1.4)
Sex	
Male	56 (75.7)
Female	18 (24.3)
Cell type	
Squamous cell	15 (20.3)
Adenocarcinoma	31 (41.9)
Large cell undifferentiated	8 (10.8)
Undifferentiated	3 (4.1)
Other non-small cell	5 (6.8)
Data not available	12 (16.2)
Disease evaluability	
Measurable	61 (82.4)
Evaluable	13 (17.6)
Response†	
Complete	0 (0)
Partial (> 50%)	4 (5.6)
Improvement (< 50%)	6 (8.4)
Stable disease	12 (16.9)
Progressive disease	49 (69.0)
Vital status	
Alive	55
Dead	19
Toxicity	
Nausea, vomiting	12 (16.2)
Leukopenia (wbcs/mm^3)	
1000–3000	22 (29.7)
< 1000	2 (2.7)
Thrombocytopenia (platelets/mm^3)	
20,000–50,000	7 (9.5)
< 20,000	4 (5.4)
Hematocrit (decrease)	
> 10%	13 (17.6)
> 15%	8 (10.8)

*AZQ dose: 30 mg/m^2 every 21 days; escalation to 35 mg/m^2.
†Response not evaluable in 3 patients.

ditional interest in clinical trials of AZQ as a single agent in the treatment of lung carcinoma.

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Use of the KB Cell Line for In Vitro Cytotoxicity Assays

Since the 1960s the National Cancer Institute (NCI) and others have used the KB cell line (1) for cytotoxicity assays required in the development of new anticancer compounds. The KB line was selected in preference to other available cell lines because of its human origin and because simple biochemical endpoints suitable for large-scale testing had been developed with this cell line (2). With the development of improved techniques for identifying cells in culture, it became apparent that the cell line in use as KB was, as were several other widely used lines, derived from the HeLa cell line. As early as 1967, Gartler (3) indicated this to be the case on the basis of isoenzyme analysis. With the development of chromosome-banding techniques in the early 1970s, the situation was firmly established by the demonstration of chromosome markers specific to the HeLa cell line in KB cells in use in several laboratories (4,5). Cells currently maintained at the NCI Tumor Bank and the American Type Culture Collection designated as Kb share chromosome markers and isoenzyme patterns with the HeLa cell line. As described by Gartler, the extent to which the HeLa derivation interferes with experimental work depends on the particular application. It is important to note that, in general, the KB assay has not been used by NCI for the direct selection of new compounds for development to clinical trial. For the most part, it has been used as a bioassay for following the active moiety during the fractionation process leading to the isolation of antitumor agents from in vivo active crude natural product extracts (6). As a substrate for cytotoxicity testing, the cells maintained by NCI have proven to be stable in response to treatment over a considerable period of time. In tests performed using standardized protocols (7), ED50 values for 6-mercaptopurine and other synthetic compounds have been quite reproducible. Figure 1 summarizes the results for 6-mercaptopurine over an interval of > 20 years. Because of the stability of the response of the cell line and the large amount of data available from prior testing, we feel that KB remains an excellent cell type for in vitro cytotoxicity testing in spite of the convincing evidence for its derivation from HeLa.

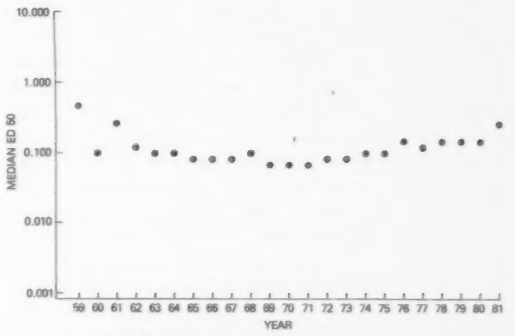

FIGURE 1.—Median ED50 values for 6-mercaptopurine in the KB system are presented for the yrs 1959–1981. More than 2000 individual assays were performed by 13 independent laboratories using NCI protocols. No indication of a significant change in sensitivity to 6-mercaptopurine is observed over this 22-yr period.

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Intermittent Antiestrogen Therapy in Advanced Breast Cancer

The following case history is, to my knowledge, the first report of locoregional metastases from breast cancer to be successfully treated by tamoxifen (Nolvadex) in three successive courses of treatment. The tumor is at present under control by the third course, $7^1/_2$ years after antiestrogen therapy was first instituted.

Case history.—A 71-year-old female patient presented in 1967 with a large ulcerated tumor in the right breast, which had grown slowly over the previous 5 years. Simple mastectomy was carried out but no attempt was made to remove multiple fixed nodes in the right axilla. Postoperatively, a course of cobalt therapy was given to the chest wall, axilla, and supraclavicular areas. In 1973, the patient presented with massive edema of the right arm and a large mass of right axillary nodes ulcerating through the skin. Premarin (1.25 mg three times daily) was given for 5 months, but there was no evidence of tumor regression and new skin nodules appeared above the mastectomy scar. In October 1974, tamoxifen (10 mg twice daily) was prescribed and taken for $2^1/_2$ years. Complete regression of the axillary mass and skin nodules occurred and persisted for another $1^1/_2$ years before the skin nodules reappeared. In April 1979, after a treatment-free gap of 2 years, tamoxifen (20 mg twice daily) was again prescribed and taken for $1^1/_2$ years. The nodules disappeared for 1 year and then reappeared. In October 1981, after a further treatment-free gap of 1 year, tamoxifen (20 mg twice daily) was prescribed and has caused regression of the skin nodules for 12 months to date. The patient is now 86 but in good general condition and active despite massive edema of the right arm.

Antiestrogen therapy for advanced breast cancer is given continuously in most centers and is maintained until tumor regrowth is clinically overt. In the past, when high-dose estrogen or androgen therapy was used in such cases, it was common to suspend treatment as soon as regression of obvious lesions was achieved, because of the unpleasant side effects usually associated with these agents. I have reported a patient who received three distinct courses of ethinyl estradiol, resulting in control of soft tissue metastases for a total of 6 years (1).

It is possible that intermittent administration of tamoxifen may postpone the ultimate development of tumor autonomy in advanced breast cancer. Both in cytotoxic and additive hormonal therapy in this disease, the present clinical practice is to persevere with an initially effective agent (or combination) until overt evidence of recurrence. However, Schabel et al (2) have suggested that the agent be replaced long before that time because experimental evidence shows that overprolonged use of an effective (but not curative) treatment is merely encouraging overgrowth by a mutant drug-resistant cell population.

In a patient receiving tamoxifen therapy, the nadir of tumor burden in the body is reached long before clinically overt reactivation is apparent. After that point, the net number of sensitive cells killed by the ongoing hormonal treatment is low, while the resistant cells proliferate and replace the growing tumor population. That is why it is rare to see a second response to an agent continued until overt tumor reactivation. What we desperately need is a biologic marker that can indicate when the nadir of tumor burden is reached, so that useless treatment is not continued.

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Angina Pectoris After Doxorubicin and Mitomycin Therapy

Antineoplastic agents have a wide variety of cardiac toxic effects ranging from transient arrhythmias to severe congestive heart failure (CHF) (1). We recently cared for a patient who developed angina pectoris with ischemic changes on ECG shortly after receiving doxorubicin and mitomycin. This toxic effect had not been previously described for either of these agents.

A 38-year-old Korean-born woman with advanced

gastric adenocarcinoma was admitted to the hospital for the management of uncontrolled nausea and vomiting. Symptomatic treatment was initiated, but when the symptoms (secondary to ascites and hepatic metastases) could not be effectively controlled, the patient consented to a trial of a second course of chemotherapy with 5-FU, doxorubicin, and mitomycin.

Seven weeks earlier the woman had received an initial outpatient treatment consisting of 5-FU (600 mg), doxorubicin (40 mg), and mitomycin (14 mg) by iv injection. Two days later she contacted her physician because of a 2-day history of retrosternal chest discomfort and fever. When seen in the emergency room she appeared to be in moderate distress, but no specific cardiac or pulmonary etiology of the chest pain could be discerned. She was admitted to the hospital for observation and evaluation of the fever. No cause of fever, other than hepatic metastases, was identified. A second injection of 5-FU was given without incident. Although there was some transient objective improvement in the patient's condition, she refused further chemotherapy until the present admission.

The patient received doxorubicin (40 mg) and mitomycin (14 mg) by iv injection at 2 PM. At 4 PM she called the nurse because of severe and crushing retrosternal chest pain with associated shortness of breath which she stated began shortly after her chemotherapy injection. The house officer was called and the patient was evaluated and given sublingual nitroglycerin which relieved the pain within 5 minutes. The chest pressure recurred at 30 minutes, and an ECG at that time showed T-wave inversion in leads II and V_3 through V_6. Compared to the patient's admission tracing, the inversions in leads II and V_4 through V_6 were new, with the V_3 T-wave inversion being more pronounced. She was subsequently treated with nitroglycerin paste, morphine sulfate, and nasal oxygen with some improvement, but the chest pains recurred, lasted until the next morning, and then subsided. An ECG taken approximately 7 hours after the chemotherapy administration showed persistent but improving T-wave inversions. On the subsequent morning, the T waves in V_5 and V_6 were flat and by the next day had returned to an upright position. Total creatine kinase and CK-MB drawn at two times during the 24 hours following the chest pain were within normal limits. Subsequently, 5-FU chemotherapy was given by continuous infusion without chest pain or ischemic changes on ECG. An echocardiogram showed a small pericardial effusion, but otherwise was within normal limits. The patient was a nonsmoker with no prior history of cardiac symptoms or risk factors for coronary artery disease.

The most serious toxic effect associated with doxorubicin administration is myocardial degeneration, which subsequently leads to refractory CHF (2). Other well-documented cardiac findings include transient ECG changes, transient symptomatic arrhythmias, and diminished QRS voltage which may be related to the cumulative drug dose (1). While age and pre-existing heart disease seem to be risk factors for these problems, we could find no report of new-onset angina pectoris in association with doxorubicin administration. Mitomycin has been reported to cause myocardial changes similar to those of radiation-induced cardiac injury and may potentiate the risk of doxorubicin-induced CHF (1); however, it has not been reported to cause angina pectoris. In contrast, 5-FU has been reported to cause angina pectoris and even myocardial infarctions (1,3). While angina associated with 5-FU is rare, it has been seen in patients with and without pre-existing heart disease.

Because of the temporal relationship between this patient's episode of angina and ischemic ECG changes, either doxorubicin or mitomycin must be implicated as the causative agent. Ischemic changes have been noted on ECG after doxorubicin administration, but angina has not been previously reported. In retrospect, the patient's initial episode of chest pain after her first course of chemotherapy was probably ischemic in nature; however, by the time she presented for evaluation her ECG was unremarkable. 5-FU, which has been reported to cause angina, was administered alone on two occasions without difficulty.

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Acute Leukemia Developing After Intensive Chemotherapy for Small Cell Cancer of the Lung

Significant advances have lately been made in the treatment of small cell cancer of the lung (1,2). With the advent of longer survival resulting from the use of intensive therapy, reports of acute leukemia or preleukemic conditions developing as a result of intensive treatment in patients with small cell carcinoma of the lung are beginning to emerge (3–5). In the context of the therapeutic gain achieved in this disease, the risk of preleukemia and acute leukemia developing as a result of long-term intensive therapies raises concern. We report another patient who, following intensive chemotherapy for small cell carcinoma of the lung, developed acute myelogenous leukemia, and we discuss the implications of the use of long-term chemotherapy following complete remission of small cell carcinoma of the lung in the context of its benefit and the risk of second malignancy.

CASE REPORT

A 47-year-old woman was found to have a large mass in the upper lobe of the left lung following several episodes of hemoptysis. A biopsy of the lesion led to the diagnosis of small cell carcinoma of the lung. A metastatic workup revealed no clinical evidence of spread of cancer to the regional lymph nodes or to any distant sites. The patient began induction combination chemotherapy consisting of cyclophosphamide (1500 mg/m^2 on Days 1 and 22), lomustine (70 mg/m^2 orally on Day 1), and methotrexate (15 mg/m^2 orally every 2 weeks). She achieved complete regression of the tumor following the induction therapy.

The patient subsequently received maintenance chemotherapy consisting of cycles of doxorubicin (60 mg/m^2 on Days 1 and 22), vincristine (1.4 mg/m^2 on Days 1 and 22), and procarbazine (100 mg/m^2 orally on Days 1–10 and 22–32) alternating with cyclophosphamide (500 mg/m^2), lomustine (50 mg/m^2), and methotrexate (10 mg/m^2). Chemotherapy doses were adjusted according to myelosuppression, gastrointestinal toxic effects, etc. After receiving chemotherapy for nearly 15 months, the patient developed persistent pancytopenia (wbc count in the range of 1200–1800/mm^3, platelet count in the range of 50,000–70,000/mm^3, and hemoglobin level in the range of 9–10.5 g/100 ml). An attempted bone marrow aspiration was unsuccessful, but the biopsy revealed areas of variable cellularity and no evidence of tumor invasion. Chemotherapy was discontinued since the myelosuppression continued without recovery.

The patient remained clinically free of tumor, but chronic myelosuppression gradually progressed over the next 6 months downwards, with marked anemia and persistent leukopenia and thrombocytopenia (wbc count, $\leq$ 1000/mm^3; platelet count, 20,000–30,000/mm^3; and hemoglobin level, between 6 and 8 g/100 ml). The patient required frequent blood transfusions initially approximately every 4 weeks, then every 2–3 weeks. Several bone marrow biopsies showed marked dyserythropoietic changes with areas of hypocellularity, focal areas of hypercellularity containing immature megaloblastic elements involving the myeloid as well as the erythroid series, ringed sideroblasts, and moderate amount of collagen deposition. The clinical picture was considered to be consistent with a preleukemic phase. Twenty months following initial diagnosis of carcinoma of the lung and 7 months after cessation of chemotherapy, the appearance of immature blastlike cells was noted in the peripheral blood. Another bone marrow biopsy was consistent with the diagnosis of acute myelogenous leukemia. The patient died shortly thereafter, following a course of chemotherapy consisting of cytarabine and 6-thioguanine. An autopsy revealed no evidence of small cell carcinoma of the lung and confirmed the diagnosis of acute myelogenous leukemia.

DISCUSSION

The clinical picture consisting of the evolution of acute leukemia following chronic and progressive pancytopenia and abnormal bone marrow (characterized by variable cellularities, maturation arrest, dyserythropoietic features, ringed sideroblasts, etc) after long-term chemotherapy for small cell carcinoma of the lung in this patient is essentially similar to those cases reported by others (3,4). The purpose of this communication, besides recording another case of leukemia arising in a patient receiving intensive chemotherapy for small cell carcinoma of the lung, is to draw attention to the rapidity with which the leukemia developed in this case and to raise concern over the practice of long-term "maintenance chemotherapy" to achieve remission maintenance and potential cure for small cell cancer of the lung.

The development of acute leukemia following cytotoxic chemotherapy is considered to be a "late complication." Although the latency period between initiation of therapy and the development of acute leukemia is broad, the average latency period has been calculated to be around 4–5 years (2,6,7). In this context, the development of acute leukemia in this patient 20 months following initiation of chemotherapy is disturbing. While a safe threshold of chemotherapy for any malignant disorder is unknown, it appears that the risk of leukemia as a second cancer is substantially higher with: (*a*) com-

bined irradiation and chemotherapy; (*b*) combination chemotherapy using several different mutagenic and/or leukemogenic agents; or (*c*) long-term use of single mutagenic and/or leukemogenic compounds or their combination. The intensive use of chemotherapy to achieve complete remission or cure of small cell cancer of the lung seems justifiable. The value of maintenance chemotherapy for months to years following complete remission in this disorder is, however, yet to be established. Since the risk of acute leukemia is real in patients living longer following cytotoxic chemotherapy for small cell carcinoma and since many develop the preleukemic syndrome or frank leukemia while they are in complete remission of their original cancers, a moratorium on the practice of long-term maintenance chemotherapy with leukemogenic agents would appear to be a prudent course unless and until the value of such a maintenance regimen is clearly established. This may not eliminate the risk altogether, but this would at least eliminate one of the leukemogenic factors from the treatment plan of a potentially curable malignant disorder.

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Human Mammary Tumor Growth Promotion by Medroxyprogesterone Acetate in the Tumor Stem Cell Clonogenic Assay

The human tumor stem cell clonogenic assay (HTSCA), as described by Hamburger and Salmon (1), holds the promise of allowing individually selected cancer chemotherapy. Its predictive value appears to be excellent for resistance and fair for sensitivity. In addition, growth promotion can be detected, as illustrated by the following observation.

A 47-year-old female had massive lymph node, bone, and mesenteric metastases of mammary carcinoma. The primary tumor and a lymph node metastasis had low but significant estrogen receptor levels of 14 and 16 fmols/mg of tissue protein, respectively. The disease had progressed after ovariectomy, standard combined hormone therapy and chemotherapy, and phase II chemotherapy with cisplatin and vindesine. The patient then received 900 mg/day of medroxyprogesterone acetate (MPA) orally for 1 month. While all other metastatic sites remained stable, pre-existing inguinal nodes abruptly and painfully swelled. The drug was withheld, and 2 weeks later these nodes were biopsied so that potentially useful treatment could be tested in the HTSCA. Since the lymph nodes were grossly hemorrhagic and tumor material was scanty, only ellipticinium and MPA could be tested in culture and no receptor studies were done. The fact that the node swelling appeared to be due to hemorrhage rather than to disease progression led to the resumption of MPA treatment. After 1 month, MPA administration was definitively stopped because of disease progression in the bone and soft tissue lesions. At the same time, the HTSCA results indicated growth promotion (table 1) by MPA at concentrations achieved in vivo; the patient's serum contained an MPA level of 28 ng/ml, as determined according to Kaiser et al (2). MPA withdrawal did not result in an in vivo response.

This observation confirms that hormonal sensitivity, in this case to MPA, can be detected in the HTSCA (3). It further shows that sensitivity may appear not only as colony inhibition but also as in vitro growth promotion. Since the patient had progressive lesions while

TABLE 1.—In vitro tumor stem cell growth promotion by MPA in the HTSCA*

MPA concentration (μg/ml)	No. of colonies formed
0	1 ± 1
0.01	6 ± 2
0.05	8 ± 1
0.20	36 ± 7
1.00	43 ± 10

*A monocellular suspension containing 1.2×10^5 trypan blue-negative cells was obtained from 1 g of tissue. Cells were cultured in a semisolid agar medium according to standard procedures (ref 1) and in the continuous presence of increasing concentrations of MPA. Untreated controls and the samples subjected to 1-hr pulse treatment with ellipticinium failed to show colony growth after 4 wks, whereas at increasing concentrations of MPA a dose-dependent induction of tumor stem cell proliferation was observed.

being treated with MPA, the clinical evolution is consistent with the in vitro observation. However, in this clinical case, in vivo growth promotion by MPA treatment cannot be considered as proved, since this would require unequivocal demonstration of acceleration of the growth of measurable lesions. Colony formation was conditioned by MPA, which suggests that in some HTSCAs that yield no colony growth, the culture medium may be devoid of a hormone(s) on which tumor growth is dependent in vitro and perhaps in vivo.

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AMSA-Induced Seizures in Patients With Hypokalemia

The effectiveness of AMSA in treating both acute nonlymphoblastic leukemia and acute lymphoblastic leukemia is well-established (1,2). Rarely, patients receiving AMSA have developed cardiac dysrhythmias (3–5); seizures, temporally related to the administration of the drug, have also been described (4). We report here the occurrence of seizures in two patients receiving AMSA and, as in the case of dysrhythmia, propose an association with electrolyte disturbance.

CASE REPORTS

Patient 1

Patient 1 was a 45-year-old female with a diagnosis of acute nonlymphoblastic leukemia established in December 1978. She was treated with cytarabine (ara-C) and doxorubicin and achieved a complete remission. One year later she was admitted with sepsis and recurrent leukemia. After initiation of appropriate antibiotics, she began combination chemotherapy that included AMSA (AAT) as described previously (5). On January 16, 1980, she received AMSA at a dose of 225 mg/m^2. One hour following the first dose she was found to be unresponsive, with a blood pressure of 110/70 and a pulse of 96; 5 minutes later she had a grand mal seizure. The serum potassium on January 15 was 2.8 mEq/L, and the electrolytes at the time of her seizure were sodium of 139 mEq/L, potassium of 3.0 mEq/L, chloride of 98 mEq/L, and CO_2 of 17 mEq/L. Magnesium was 0.8 mEq/L, phosphorus was 0.6 mEq/L, glucose was 98 mg/dl, and calcium was 8.1 mg/dl. A lumbar puncture was positive for leukemic cells. An ECG showed ventricular tachycardia, and the patient was cardioverted with iv lidocaine. Despite replacement with iv potassium, serum potassium continued in the range of 2.7–3.5 mEq/L. The patient received her second dose of AMSA on January 17, 1980. She again developed tachycardia followed by grand mal seizures. She progressively deteriorated, and died 4 days later.

Patient 2

Patient 2 is a 60-year-old female who was referred for treatment of acute promyelocytic leukemia. She was treated with daunorubicin, ara-C, thioguanine, and iv heparin to control disseminated intravascular coagulation. Ten days after the completion of treatment a bone marrow aspiration confirmed persistent leukemia. She then began treatment with AMSA (160 mg/m^2 iv every day × 3), ara-C (160 mg/m^2 by continuous infusion every day × 5), and thioguanine (100 mg/m^2 every 12 hours). She tolerated the first dose of AMSA without difficulty. One hour after receiving the second dose of AMSA, she developed grand mal seizures that were controlled by dilantin. An ECG was not obtained at that time. All diagnostic studies (including a computerized tomographic scan) failed to reveal the origin of the seizures. The serum electrolytes on Day 1 of AMSA were sodium of 143 mEq/L, potassium of 3.4 mEq/L, CO_2 of 28 mEq/L, and chloride of 96 mEq/L. On the day that the patient received her second dose, the serum sodium was 137 mEq/L, potassium was 2.9 mEq/L, CO_2 was 28 mEq/L, and chloride was 96 mEq/L. The blood sugar was normal. In view of the progressive hypokalemia, the third and final dose of AMSA was withheld until the potassium was restored to 4.8 mEq/L. The patient then received the third dose successfully without any untoward effects.

DISCUSSION

Dysrhythmias related to the administration of AMSA have been reported, but in all instances this has been associated with concurrent hypokalemia. Only four cases of seizures temporally related to the administration of AMSA have been reported. In all but one instance (4), there was documentation of hypokalemia implying that, as in the case of dysrhythmias, this may be a predisposing factor for the development of AMSA-related seizures. In our patient who developed seizures after administration of AMSA, the drug was given safely when the serum potassium level was restored to normal.

Experience with these two patients further supports the conclusion that the administration of AMSA is contraindicated in patients with hypokalemia. Since neither dysrhythmias nor seizure activity has occurred in patients with serum potassium values of > 2.9 mEq/L, a reasonable recommendation allowing for a sufficient safety margin might be a minimum serum potassium value of 3.5 mEq/L. It is also insufficient to assure an adequate serum potassium value only on Day 1 of a treatment course. Since patients receiving iv fluids and antibiotics may have considerable variation in their serum electrolytes from day to day, it is inappropriate to administer the drug without documentation of an adequate serum potassium on the day the drug is given.

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Topics in Emergency Medicine

San Francisco, California, January 31–February 4, 1983

This course will be presented by Extended Programs in Medical Education, University of California School of Medicine, San Francisco and will take place at the Sheraton Hotel at Fisherman's Wharf. The program is designed to instruct emergency medical practitioners in depth through didactic presentations, case studies, student/teacher interactions, and demonstrations in the approach to problems in the emergency department. Upon completion of this program, registrants should be more competent in handling injuries of the hand and face, in diagnosing and treating acute cardiac disease, in interpreting radiographs, and in managing problems in other areas including pediatrics, ophthalmology, neurology, obstetrics/gynecology, and endocrinology. Pre- and post-course assessments will be available, as well as a syllabus with references.

This course meets the criteria for 35 hours of credit in the following: Category 1 of the AMA Physician's Recognition Award, the CMA Certification Program, the American Academy of Family Physicians, and Category 1 of the American College of Emergency Physicians.

For further information, contact: Extended Programs in Medical Education, University of California, Rm 569-U, Third and Parnassus Ave, San Francisco, CA 94143 (telephone 415-666-4251).

Roswell Park Memorial Institute Continuing Education Programs

Buffalo, New York, February–June 1983

Roswell Park Memorial Institute is conducting monthly continuing education programs in oncology for community practitioners to disseminate information on the newest methods in diagnosis and treatment of cancer. Each monthly seminar is organized by a different department of the Institute covering a different clinical specialty. These seminars qualify for Category I credit toward the AMA Physician's Recognition Award, prescribed credit by the American Academy of Family Physicians, and Category 2-D credit by the American Osteopathic Association. There is a $45.00 registration fee for one day seminars and a variable fee for longer programs. The seminars are cosponsored by the Cancer Control Program of the Institute and the American Cancer Society, New York State Division and Erie County Unit.

The seminars are scheduled as follows: Ovarian Cancer: New Approaches to Treatment of Adults and Adolescents, *February 19, 1983* (Chairperson: Dr. M. Steven Piver; Update in Malignant Melanomas, *March 10, 1983* (Chairperson: Dr. C. P. Karakousis); Human Genetics and Cancer, *March 17, 1983* (Chairperson: Dr. Thomas Shows); Role of the Laboratory in Management of Cancer, *April 14, 1983* (Chairperson: Dr. John Fitzpatrick); Clinical and Basic Aspects of Breast Cancer, *May 12, 1983* (Chairperson: Dr. Thomas Dao); Modern Management Concepts in Leukemia and Lymphoma, *May 19, 1983* (Chairperson: Dr. Edward Henderson); Disciplinary Approach to Adolescent Oncology, *June 16, 1983* (Chairperson: Dr. Cameron Tebbi).

For further information, contact: Gayle Bersani, RN, Cancer Control Coordinator, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716-845-4406).

International Lung Cancer Update Conference

New Orleans, Louisiana, March 3–5, 1983

A panel of international lung cancer experts will present the latest information on this worldwide problem, which has now reached epidemic proportions. The meeting is oriented toward an analysis of lung cancer epidemiology and is aimed at gaining insight into prevention and lung cancer risk factors: smoking, industrial exposure, asbestos, nutrition, the interaction of the different factors, etc. This meeting will also include a comprehensive review of the most recent advances in lung cancer biology and the use of this new knowledge to resolve fundamental clinical problems.

This conference has been organized to present the most up-to-date information about these developments to practicing physicians, house staff, nurses, basic scientists, medical and graduate students, and all health professionals involved with cancer patients.

For additional information, contact: Dr. Merle Mizell, Laboratory of Tumor Cell Biology, Tulane University, New Orleans, LA 70118.

Third Annual Current Approaches to Radiation Oncology, Biology, and Physics

San Francisco, California, March 9–11, 1983

Through a syllabus and a series of lectures, this course will include present knowledge and advances in the field of radiation oncology, biology, and physics. The program will be of interest to everyone in the field including students. Presentation of the annual Franz Buschke Prize and the Franz Buschke Lectureship, featuring Dr. Herman D. Suit of Harvard Medical School, will highlight the course.

This program presented by the Department of Radiation Oncology of the University of California School of Medicine in San Francisco, is sponsored by Extended Programs in Medical Education. The course will meet the criteria for AMA/CMA Category 1 (formal) continuing education credit for physicians. Continuing education credit for nurses and radiation technologists will also be provided.

For further information, contact: Extended Programs in Medical Education, University of California School of Medicine, Rm 569-U, Third and Parnassus, San Francisco, CA 94143. Telephone (415) 666-4251.

Sixth Annual Mid-Willamette Valley Cancer Conference

Gleneden Beach, Oregon, March 11–12, 1983

The sixth annual Mid-Willamette Valley Cancer Conference will be held at the Salishan Lodge in Gleneden Beach, Oregon. The conference will feature a lecture series given by guest speakers from all over the country. The topic of discussion is lung cancer and will include an overview, clinical features, diagnosis and staging, surgical management, radiation therapy, chemotherapy, and immunotherapy of the disease.

The conference qualifies for AMA accreditation. Salishan Lodge has asked that reservations for room and board be made early (telephone 503-764-2371).

For more information, contact: Mrs. Beverly Kay, Executive Secretary, Salem Hospital CME, PO Box 14001, Salem, Oregon 97309-5014 (telephone 503-370-5339, M-F, 7:00 am-3:00 pm).

Pulmonary Diseases and Critical Care Medicine: A Practical Approach

Park City, Utah, March 12–19, 1983

This program is designed for general internists, family practitioners, general practitioners, pulmonologists, and anesthesiologists. Much of the information covered will also be valuable to physician's assistants, nurses, and respiratory therapists.

The purpose of the program is to provide a practical approach to the evaluation and management of patients with pulmonary diseases, including those who are critically ill.

There will be five sections: environmental and immunologic lung diseases, infectious lung diseases, applied respiratory physiology, diseases of the airways and pulmonary vasculature, and acute respiratory failure. The sections consist of four lectures, followed by a panel discussion and question-and-answer period with all the faculty. The informal atmosphere of the program and the intense participation of the faculty should provide a valuable educational experience for all who attend. A syllabus outlining the lecture topics and containing review articles will be provided at the program.

Program participants will learn to use simple pulmonary function tests in diagnosing and caring for patients, differentiate the various kinds of pulmonary edema and treat them appropriately, apply new antibiotics and bronchodilator drugs, evaluate patients with asbestosis and other pneumoconioses, and manage patients with acute respiratory failure in an intensive care setting.

This program meets the criteria for 19 hours in Category 1 of the Physicians Recognition Award of the AMA and the Certification Program of the CMA, 19 prescribed hours of the American Academy of Family Practice, and 19 hours in Category 1 of the American College of Emergency Physicians.

For further information, contact: Extended Programs in Medical Education, University of California, 1456 Ninth Ave, San Francisco, CA 94122. For registration information, call (415) 666-5808. For program information, call (415) 666-4251.

Seventh Annual Meeting of the American Society for Preventive Oncology

Bethesda, Maryland, March 24–25, 1983

The seventh annual meeting of the American Society for Preventive Oncology will be held at the Holiday Inn in Bethesda, Maryland.

For further information, contact: Dr. David Schottenfeld, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

Two Epidemiology Courses

San Francisco, California, April 6–8 and 9–10, 1983

Two courses sponsored by the New England Epidemiology Institute will take place at the Cathedral Hill Hotel. The first course, Epidemiology Methods and Applications, will be presented by Drs. Philip Cole (University of Alabama) and Kenneth Rothman (Harvard University) on April 6–8. The instructors will present modern concepts in epidemiology and their applications to the study of etiology, natural history of disease, and strategies in preventive medicine and public health. The course is especially appropriate for those who evaluate and interpret epidemiologic research, but is intended for professionals in health and related disciplines who wish to develop a familiarity with epidemiologic research principles.

The second course, Clinical Epidemiology, will be presented by Drs. Noel Weiss and Larry Koepsell on April 9–10. The instructors will present an overview of epidemiology and its application to decision making in the clinical setting, and the methods used to evaluate diagnostic and therapeutic regimens, the application of these methods, and their strengths and limitations. Lectures that set forth the principles and methods of clinical epidemiology will alternate with problem-solving sessions and discussions that are intended to apply the didactic material to situations encountered in medical practice.

No previous study of epidemiology or biostatistics for either course is required. The courses qualify for AMA Category 1 credit and/or certification maintenance credits from the American Board of Industrial Hygiene.

For more information, contact: Dr. Nancy Dreyer, New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Epidemiology Summer Program

Amherst, Massachusetts, July 31–August 20, 1983

The University of Massachusetts will host a 3-week summer program in Epidemiology at the Amherst campus in western Massachusetts. The program includes both methodologic and substantive courses, and is designed to be of interest to those seeking an introduction to concepts in epidemiology as well as those desiring a review of recent developments in epidemiologic thinking. Courses offered include: Epidemiologic Methods, Introductory Biostatistics, Multivariate Methods in Epidemiology Research, Clinical Epidemiology, Cancer Epidemiology, Cardiovascular Disease Epidemiology, the Epidemiology of Reproductive and Congential Disorders, and Biomedical Writing.

Invited faculty include teachers and prominent researchers from leading universities. The course qualifies for credits toward graduate degrees, continuing medical education, or certification maintenance by the American Board of Industrial Hygiene.

For further information, contact: Dr. Nancy Dreyer, New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167.

Current Concepts in Cancer Therapy, II

St. Louis, Missouri, November 3–5, 1983

This meeting will be presented by the Department of Surgery and the Office of Continuing Medical Education, Washington University School of Medicine in St. Louis. The program chairman is Marc K. Wallack, MD. The meeting will be accredited for 17 hours AMA Category 1, AAFP Prescribed, and AOA.

For further information, contact: Loretta Giacoletto, Office of CME, Washington University School of Medicine, Box 8063, 660 S Euclid, St. Louis, MO 63110 (telephone 314-454-3873).

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